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MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
Division of Clinical and Behavioral Research,
Division of Biological and Biochemical Research, and
Division of Special Mental Health Research

United States. NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1980 - September 30, 1981

VOLUME II

INDIVIDUAL PROJECT REPORTS

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ANNUAL REPORT
MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
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NATIONAL INSTITUTE OF MENTAL HEALTH

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RESEARCH PROJECT SERIAL NUMBER LISTING (Cont.)

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00092-07 BP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Central Amines and Aggression, Suicide, and Alcoholism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	Gerald L. Brown, M.D.	Medical Officer	BP	NIMH
Other:	Frederick K. Goodwin, M.D.	Chief	CP	NIMH
	O.L. Royal, M.D.	Chief, Department of Psychiatry		NNMC
	William E. Bunney Jr., M.D.	Chief	BP	NIMH
	Peter F. Goyer, M.D.	Medical Officer		PNMC
	William J. Klein, M.D.	Medical Officer		NNMC

COOPERATING UNITS (if any)

Clinical Psychobiology Branch, DCBR, NIMH; Department of Psychiatry, National Naval Medical Center; Portsmouth Naval Medical Center

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.85	.55	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The National Institute of Mental Health (NIMH) and the National Naval Medical Center (NNMC) collaboratively study central amine metabolites in the cerebrospinal fluid (CSF) of psychiatric patients. Results to date indicate that aggression is inversely correlated with CSF 5-hydroxyindoleacetic acid (5HIAA) and positively correlated with CSF 3-methoxy-4-hydroxyphenyl glycol (MHPG) and cyclic 3', 5'-adenosine monophosphate (c-AMP). Low CSF 5HIAA is also associated with suicidal history. Findings have been largely replicated on two separate populations. Alcoholics have an increased CSF 5HIAA. Disulfiram (Antabuse) appears to lower CSF homovanillic acid (HVA) and appears to increase serum norepinephrine (NE); low CSF dopamine-β-hydroxylase (DBH), low platelet monoamine oxidase (MAO), low plasma amine oxidase (AO) and high red-cell catechol-O-methyl transferase (COMT) are related to adverse reactions to disulfiram. CSF DBH is inversely related to significant deviations in certain personality measures on the MMPI. Within these populations neither clinical depression nor aggressive behavior is significantly related to alcoholism per se. These patients have also provided control data for other psychiatric diagnostic groups at NIH.

(Rev. 10-76)

Project Description:

Objectives: Evidence obtained in recent years indicate that epinephrine (E), norepinephrine (NE), dopamine (DA), serotonin (5HT), and gamma-amino butyric acid (GABA), among others, act as neurotransmitters and/or neuromodulators of central nervous system (CNS). Although considerable indirect pharmacologic evidence has linked these amine systems with psychiatric illnesses (particularly affective illness and schizophrenia), the relative lack of direct data in man has limited the applicability of these hypotheses to improved diagnosis and treatment of the major psychiatric disorders. Direct data from man can be immensely valuable in making use of the massive data from animals and assessing the differences and similarities between man and animals. Recent data suggests that affective disorders and psychoses may yield to biological sub-typing which would enable a more effective choice of pharmacological therapy for individual patients. There has been virtually no data on central neurochemical function in the various personality disorders--a rather striking deficit in our knowledge, considering the evidence suggesting that some personality disorders, particularly those involving criminality, have patterns of a genetic component. Data from animals suggests a relationship between aggressive behavior and biogenic amines. Similarly, human data also suggests a genetic component in suicidal behavior. Neurochemical studies in human alcoholism have also been limited. A purpose of this project is to extend the studies of central amine turnover into larger and more diverse populations of psychiatric patients.

Methods Employed: Techniques and procedures for obtaining, storing, and analyzing CSF for various neurochemicals have been developed at NIH and elsewhere. Some techniques (i.e., probenecid) allow for an estimate of brain amine turnover. Other studies in conjunction with precursor and pharmacological interventions have further provided knowledge of functional brain chemistry in relationship to behavior, diagnosis, and personality. These techniques have proven to be relatively innocuous over several years.

Major Findings: Findings suggest that a group of personality disorders with problems secondary to poor impulse control, high levels of anger-hostility, and poor judgment, when compared to a mixed control group from NIMH (normals and patients with non-CNS neurological disorders), have similar baseline 5-hydroxyindoleacetic acid (5HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG). Furthermore, results to date indicate that aggressive behavior may be inversely correlated with 5HIAA and positively correlated with MHPG. A group of personality disorders have also shown no statistically significant difference in CSF cyclic 3', 5'-adenosine monophosphate (c-AMP) from neurological patients with non-CNS disorders or from groups of depressive, manic, and schizophrenic patients, though aggressive behavior is positively correlated with c-AMP. Those patients who were administratively discharged from the service and those with prior history of

suicidal attempts have shown lower CSF 5HIAA and higher MHPG, c-AMP and c-GMP. A second group of patients with borderline personalities (DSM-II) show an inverse relationship between CSF 5HIAA and psychopathic deviation (MMPI) as well as history of aggressive behavior. The relationship between CSF 5HIAA and suicidal behavior was also similar in this latter group.

A group of alcoholics have also been studied. It has been found that alcoholics do not differ from personality disorders with respect to HVA. However, studies do indicate that the mean 5HIAA is higher in the intoxication withdrawal stage and decreases over time in relation to the length of abstinence to reach a baseline value not differing from the personality disorders. An additional study shows that, though HVA values do not change in relationship to the time period post intoxication-withdrawal, they are depressed concomitant with the use of disulfiram (Antabuse), a dopamine-β-hydroxylase (DBH) inhibitor. In addition, disulfiram use correlates with a significant increase in serum NE. Mean serum DBH in alcoholics versus normal controls was significantly lower, blood pressure was significantly higher, and serum NE was not different. Disulfiram is also associated with an increase in cholesterol in alcoholics. Other studies indicate that lower DBH is correlated with increasing psychopathology as measured by the Minnesota Multiphasic Personality Inventory (MMPI) and lower CSF DBH is associated with disulfiram-induced psychoses. Furthermore low platelet monoamine oxidase (MAO), low amine oxidase (AO), and elevated erythrocyte catechol-O-methyl transferase (COMT) are associated with disulfiram-induced psychoses.

Other studies show that neither clinical depression nor aggressive behavior in this group of early to mid-stage alcoholics can be associated with alcoholism. Aggressive behavior can be associated with alcoholism only when the alcoholics are also personality disorders.

Significance for Mental Health Research: CNS functioning is greatly under-studied in some major groups of psychiatric patients, viz. personality disorders, alcoholics, and borderlines. Studies of animal models, as well as Gilles de la Tourette and hyperactive children suggest a relationship between central neurotransmitter systems and aggressive behavior. Human suicidal behavior has an enormous public health and social significance and, except for some work from Sweden, has not been studied for biochemical contributions. The neurobiological aspects of alcoholism, either predisposing, concomitant, or resultant, are of timely significance as alcoholism is a prevalent problem. Also drug-free personality disorders may serve as a useful comparison group for biological studies of other psychiatric disorders.

Proposed Course of Project: The preparation for this project began in January 1973. The approval processes, both in terms of scientific merit and the protection of rights of patients, were completed in July 1974. The first lumbar puncture was performed in September 1974. This progress of the project is submitted to the Navy for reapproval each March. We believe this collaboration continues to be of mutual benefit to NIMH and NNMC. Some of the publications cited below include authors who were collaborators in the past, but are no longer associated with the ongoing collaboration.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00021-16 BP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Sleep

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. C. Gillin	Research Psychiatrist, Unit on Sleep Studies	BP	NIMH
OTHER:	W. B. Mendelson	Research Psychiatrist	SMR	NIMH
	R. J. Wyatt	Chief, Lab. of Clin. Psychopharmacology	SMR	NIMH
	R. J. Loewenstein	Clinical Associate	BP	NIMH
	W. E. Bunney, Jr.	Chief	BP	NIMH
	D. L. Murphy	Chief	CN	NIMH
	F. K. Goodwin	Chief	CP	NIMH
	R. M. Post	Chief, Sec. on Psychobiology	BP	NIMH
	E. S. Gershon	Chief, Sec. on Psychogenetics	BP	NIMH

- See Attached Continuation -

COOPERATING UNITS (if any)

Adult Psychiatry Branch, NIMH
Division of Special Mental Health Research, St. Elizabeths Hospital, Wash. D.C.
- See Attached Continuation -

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Sleep Studies

INSTITUTE AND LOCATION NIMH; ADAMHA; NIH; St. Elizabeths Hospital, Wash. DC 20032
NIMH, ADAMHA; NIH; Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.0	3.0	10.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This unit, which is jointly administered under W. E. Bunney, Jr., Chief, Biological Psychiatry Branch, and Richard J. Wyatt, Chief, Adult Psychiatry Branch, has long conducted studies on basic and clinical aspects of sleep in man and animals. It has made contributions to the basic neuropharmacological control of sleep and nocturnal neuroendocrinology, clinical use and abuse of sleeping pills, affective illness, schizophrenia, insomnia, childhood enuresis and obsessive-compulsive disorder, Gille de la Tourette's syndrome, dementia, and adult obsessive-compulsive disorder. Our studies suggest that patients with primary affective illness have a supersensitive cholinergic system both when ill and when in remission.

Progress has been made in identifying an endogenous sleep factor in sleep-deprived rat brain. Local glucose utilization is down by approximately 30% throughout the brain during NREM sleep.

PHS-6040
(Rev. 10-76)

Names, Laboratory and Institute Affiliations, and Titles of Principal Investigators and All Other Professional Personnel Engaged on the Project (Cont'd):

OTHER:	H. Weingartner	Research Psychologist	LPP	NIMH
	D. P. van Kammen	Chief, Sec. on Neuropsychopharmacology	BP	NIMH
	M. H. Ebert	Chief, Sec. on Experimental Therapeutics	LCS	NIMH
	J. Rapoport	Unit on Childhood Mental Illness	BP	NIMH
	T. A. Wehr	Chief, Clin. Research Unit	CP	NIMH
	P. Gold	Chief, Unit on Neuroendocrinology	CP	NIMH
	M. S. Buchsbaum	Chief, Section on Clinical Psychophysiology	BP	NIMH
	L. Sokoloff	Chief	LCM	NIMH
	C. Kennedy	Section on Developmental Neurochemistry	LCM	NIMH
	M. Mishkin	Chief, Section on Cerebral Mechanisms	LN	NIMH
	R. Nakamura	Senior Staff Fellow	LPP	NIMH
	Saul Rosen	Senior Investigator	CEB	NIAMDD
	D. Lewy	Clinical Psychobiology Branch	LCS	NIMH
	F. C. Chu	Neuro-opthalmology Section	CB	NEI

Cooperating Units (Cont'd):

Laboratory of Psychology and Psychopathology, NIMH
Clinical Neuropharmacology Branch, NIMH
Clinical Psychobiology Branch, NIMH
Section on Psychobiology, BPB, NIMH
Section on Psychogenetics, BPB, NIMH
Section on Neuropsychopharmacology, BPB, NIMH
Section on Experimental Therapeutics, LCS, NIMH
Unit on Childhood Mental Illness, BPB, NIMH
Clinical Research Unit, CPB, NIMH
Section on Clinical Psychophysiology, BPB, NIMH
Department of Medicine, University of Rochester, Rochester, New York

Project Description:

Objectives:

Experimental findings over the past decade promise understanding of the basic biochemical processes underlying sleep; and increasingly clear demonstrations of the severity of sleep disturbances in many psychiatric patients indicate the need for better means of alleviation. To whatever extent understanding of sleep can be achieved, more effective treatment of its disturbances will surely follow.

I. Major Findings: Pharmacological Studies of Sleep

A. Cholinergic

1. Time-dependent Effects of Physostigmine on Normal Human Sleep and Arousal

Physostigmine induces REM sleep or arousal in man depending upon the dose and time of administration. Thirty-three normal volunteers received 40 physostigmine (0.5 mg over 2-3 min) and 40 matched saline (sal) infusions. (1) With postinfusion-arousals excluded, infusions given 5 min after sleep onset reduced time from infusion to onset of REM from 78.9 ± 14 min (sal) to 52.4 ± 10.4 (phy) ($p<0.05$, two-tailed "t" test). (2) Infusions given 35 min after sleep onset reduced time from infusion to onset of REM from 65.7 ± 15.2 (sal) to 11.2 ± 2.1 (phy) ($p<0.005$). REM was induced more rapidly by physostigmine infusions given 35 min after than 5 min after sleep onset ($p<0.01$). (3) For the infusions at REM onset, 5 min after and 25 min after REM, the predominant effect was one of arousal. Judging from number and duration of awakenings, the order of intensity of arousal was: REM > 5 min after > 25 min after REM. Although a dose of 0.5 mg awoke the subjects when given 25 minutes after the first REM period, a dose of 0.25 mg induced REM.

These data suggest that cholinergic mechanisms are involved in initiating REM sleep and arousal. During the first NREM period, physostigmine more readily induces REM when given 35 min than when given 5 min after sleep onset, whereas the same dose given at REM onset and during the second NREM period produces different intensities of arousal.

In order to determine whether the duration of REM could be affected, seven normal male volunteers (mean age 22.8) were studied for three nights (one "adjustment" and two experimental nights). Subjects were pretreated with 0.75 mg IM methscopolamine prior to bedtime; and during sleep they received either placebo or physostigmine 1.0 mg given by a slow IV drip over a period of 60 minutes. The IV infusion began 35 minutes after sleep onset and ended about 95 minutes after sleep onset. Physostigmine significantly decreased both the latency of the first REM period (Placebo = 108.4 ± 16.2 min (mean + S.E.) physo = 61.7 + 2.2 min, $p<.05$ and the duration of the second NREM period (placebo 110.4 ± 13.6, physo = 74.4 ± 6.5, $p<.05$). The duration of individual REM periods was not altered. Although total REM time and REM percent showed a non-significant increase of physostigmine (RT = 98.2 ± 6.4 min, R% = 25 ± 1.5%) as compared to placebo nights (RT = 80.9 ± 9.4, R% = 20.1 ± 1.9%) this can be accounted for by the greater number of REM periods on physostigmine (4.3 ± 0.3) than on placebo (3,6 ± 0.2) conditions. Cholinergic mechanisms may modulate the "timing" rather than the "maintenance" of REM.

2. Physostigmine-induced REM is Associated with Normal Dreaming

To examine the psychological process of dreaming in man, seventeen normal volunteers (11M, 6F, mean age 23), after pretreatment with methscopolamine 0.5 mg IM, slept with an IV needle, and at either 10 or 35 minutes after sleep onset received one intravenous infusion per night of either placebo or physostigmine 0.5 mg.

During Experiment I subjects were given an IV infusion 10 min after sleep onset and were awakened 20 min after infusion. Experiment II included awakening either 7 or 20 min following an infusion given 35 min after sleep onset; in addition subjects were instructed to go back to sleep after the 20 min awakening and were then awakened approximately 7 min after they had spontaneously gone into REM sleep. The above study was designed to control for (1) time of infusion, (2) time from infusion to awakening, (3) state of sleep prior to awakening.

At each awakening a taped interview was conducted. Subjects were questioned waking. After the interview subjects filled out a questionnaire. A dream mentation scale was constructed from the questionnaire ranging from +5 (absolute confidence in presence of dream) to -5 (absence of dreaming).

Comparison of Dream Reports from Physostigmine-induced and Spontaneous First REM Period

		Physostigmine-induced REM Periods	Spontaneous REM Periods	p
1)	No. of REM awakenings	9	10	NS
2)	No. of dream reports*	8	7	NS
3)	Duration of REM prior to waking (mean+SEM min)	6.6 ± 0.6	7.1 ± 0.4	NS
4)	REM Density (0-8 scale)	1.7 ± 0.2	1.7 ± 0.4	NS
5)	Vividness of dreams*	3.1 ± 0.4	2.7 ± 0.4	NS
6)	Unusualness (0-5)*	1.6 ± 0.5	1.7 ± 0.3	NS
7)	Emotionality (0-5)*	2 ± 0.4	1.9 ± 0.5	NS

* These scores were obtained from a questionnaire filled out independently by both subjects and experimenter and from a structured interview conducted by the experimenter.

During Experiment I and the 7 minute post-infusion awakenings of Experiment II, neither REM sleep nor dreaming was induced by physostigmine. In the 20 minute post-infusion, physostigmine induced the onset of REM sleep in approximately 13 minutes in all but one subject. Those drug-induced REM episodes were associated with a significantly higher incidence of dream recall than placebo.

This study indicates that the physostigmine-induced physiological characteristics of REM sleep are accompanied by dreaming. The dreaming occurring during physostigmine-induced REM periods appears to be similar to that observed during spontaneously occurring REM sleep.

3. Induction of REM Sleep in Man by Arecoline, a Cholinergic Muscarinic Agonist: Blockade by Scopolamine

Ten normal volunteers (8M, 2F, mean age 25.3) were each studied on 4 randomly ordered experimental nights (after a night of adaptation). After pretreatment with methscopolamine 0.5 IM on three of the experimental nights, they each received one intravenous infusion about 32 to 35 min after sleep onset of either (1) placebo, (2) arecoline 1.0 mg IV, or (3) arecoline 1.5 mg IV. On the fourth night pretreatment was scopolamine 0.5 mg IM followed by IV infusion of arecoline 1.5 mg. The results were: (1) Both arecoline 1.0 mg and 1.5 mg triggered the onset of REM sleep, 20.1 and 15.9 min after infusion, respectively. (2) Pretreatment with scopolamine blocked the REM-inducing effect of arecoline 1.5 mg IV. (3) Once the onset of the first REM period was moved forward, subsequent nonREM-REM cycles were advanced without altering REM-REM intervals. (4) The number of REM periods was significantly increased after arecoline 1.0 mg (4.1±0.3) and 1.5 mg (4.5±0.2) compared to placebo (3.5±0.2). (5) Total REM time and percent after arecoline 1.5 mg (100.7±6.4 min, 24.7± 1.7%) was significantly greater than placebo (76.2±5.4 min, 18.1±0.9%). The data with arecoline, a specific cholinergic muscarinic agonist, confirms our previous findings with physostigmine and suggests that cholinergic stimulation of muscarinic receptors may play a role in the triggering of REM sleep in man. In addition, the results suggest that both arecoline and physostigmine "phase advance" REM sleep towards the onset of sleep; that is, all REM periods occur earlier in the night than under normal conditions.

4. Experimental Shortening of REM Sleep Ultradian Rhythm with Timed Multiple Infusions of Arecoline

Seven normal volunteers (4M, 3F, mean age 25 yrs) were studied under the following two experimental conditions. (1) Pretreatment with methscopolamine 0.5 mg IM followed by three IV infusions of arecoline (1.5 mg, 1.0 mg and 1.0 mg doses) given at the following three times respectively: (a) 35 min after sleep onset; (b) 30 min after end of first REM period; (c) 30 min after end of second REM period. (2) Pretreatment with methscopolamine followed by three placebo (saline) IV infusions. Subjects were run either in pairs or groups of 3 such that the timing of the placebo infusions was "yoked" to the arecoline infusions.

Duration (Minutes) of NREM and REM Periods (Mean ± SEM)

	$NREM_1$	REM_1	$NREM_2$	REM_2	$NREM_3$	REM_3	$NREM_4$	REM_4	$NREM_5$	REM_5
Placebo	95.7 ±14.7	14.1 ±3.9	89.1 ±5.7	22.8 ±3.2	78.4 ±4.6	25.5 ±4.7	- -	- -	- -	- -
Arecoline	42.2* ±2.6	10.3 ±2.4	38.3* ±1.3	12.8* ±2.5	41.4* ±3.7	21.0 ±4.9	84.7 ±4.1	22.1 ±5.4 (N=6)	65.4 ±4.6 (N=6)	28.8 ±5.0

*p<.01

The results of this study are: (1) It was possible to transform a normal REM ultradian rhythm (with REM-REM interval of about 100 min) into a rhythm with REM_1-REM_2 interval of 48.6 min and REM_2-REM_3 interval of 54.2 min. (2) There were a significantly greater number of REM periods after arecoline (5±0.22) than placebo (3.71±0.29) resulting in greater REM time and percent (arecoline = 104.1±1.5.6 min, 24.8±1.9% and placebo = 84.4±4.6 min, 20.1±1.2%). (3) Duration of individual REM periods was either unchanged or shortened (e.g., REM_2). Acetylcholine plays a role in the timing of REM sleep. The increase in REM time seen appears to result from an increase in the number but not the duration of REM periods. There also appears to be a "ceiling" after which REM time cannot be further increased by cholinergic agents since both single and multiple infusions of arecoline produce the same amount of increase in REM time.

5. Acetylcholine and Sleep: Effect of Oral Choline on Normal Human Sleep

Choline, a normal dietary constituent, has recently been shown to be a precursor of brain acetylcholine. If so, in the light of our previous work with physostigmine and arecoline we would predict that choline administered to humans may alter REM sleep parameters. A double-blind crossover design with 12 normal male subjects was used. Subjects slept for 3 nights (1 adaptation and 2 experimental) and just prior to "lights out" (11:30 p.m.) were given an elixir containing either 10 g of choline chloride or placebo matched for taste, color and consistency.

Effects of Choline Chloride (10 gm) or Placebo

	Placebo	Choline (10 gm)
Total Recording Period	472 ± 5*	492 ± 4
Sleep Latency	33 ± 8	24 ± 4
Total Sleep	422 ± 8	446 ± 6
Non-REM	326 ± 10	347 ± 8
Stage I	12 ± 3	10 ± 3
Stage II	269 ± 7	273 ± 9
Stage III	22 ± 4	29 ± 4
Stage IV	25 ± 5	33 ± 5
Stage V	25 ± 5	33 ± 5
REM Sleep	95 ± 6	100 ± 5
REM Latency	92 ± 12	107 ± 12
Intermittent Awake	13 ± 4	17 ± 5
Early Morning Awake	4 ± 1	3 ± 1

* mean ± SEM
values are in minutes

The results were: (1) Choline did not have any effect on conventional sleep parameters when data from all 12 subjects were analyzed. (2) When the subjects were rank ordered with respect to their placebo (baseline) REM latency, REM time and REM %, we found that low placebo REM latencies were increased and high placebo REM latencies were decreased by choline.

The correlation between placebo (baseline) REM latency and the change in REM latency after choline (i.e., choline-placebo value) was inversely correlated $r=-0.90$, $N=12$ ($p<.01$). Similarly there was an inverse correlation between placebo REM time and REM % and change after choline ($r=0.63$ for REM time and $r=0.61$ for REM%, both $p<.05$). Whether the above changes in REM parameters produced by choline represents a genuine central neuromodulatory effect or is no more than a statistical artifact is unclear. Likewise neither choline (7.5 gm) at 8 am or 11 pm nor choline (7.5 gm) at both 8 am and 11 pm had any affect on sleep as compared with control. These results suggest choline may not enhance functional cholinergic neurotransmission, at least not in putative sleep centers.

6. The Effect of Piperidine, a Cholinergic Nicotinic Agent on Normal Human Sleep

Piperidine is an alicyclic amine with nicotine-like cholinomimetic effects on central and peripheral nervous system. In view of conflicting reports on the effect of piperidine on REM Sleep in rodents, we undertook to study the effect of oral piperidine HCl in normal volunteers. Using a 4x4 Latin square design, 8 subjects were recorded on 4 nights under the following four experimental conditions (a) placebo, (b) piperidine 200 mg, (c) piperidine 400 mg and (d) piperidine 600 mg.

All medications were given prior to "lights out" (11:30 p.m.). No change in any sleep parameter including REM latency, REM time and REM density, was observed after piperidine. Although data from animal pharmacology suggests that the doses used in our study would have central effects, the possibility still remains that physiologically effective doses were not used. Intravenous administration of piperidine during sleep is also currently underway.

7. Scopolamine-induced Muscarinic Supersensitivity in Normal Man: Changes in Sleep

An injection of scopolamine (6ug/kg) was administered on 3 consecutive mornings to normal human subjects. Sleep recordings obtained at night (when the central anticholinergic effect of the morning scopolamine was no longer present) indicated a significant reduction in latency to REM sleep onset on the nights following the second and third injection. REM latency was significantly reduced on the second (50 min $\pm$ 12) and third (45 min $\pm$ 13) scopolamine nights as compared with baseline (96 min $\pm$ 16) ($p<.005$). This effect is opposite to the direct pharmacological action of nighttime administration of scopolamine (i.e., prolongation of REM latency). Furthermore, scopolamine pretreatment on 2 consecutive mornings also potentiated the REM-inducing effect of arecoline, a central muscarinic agonist. The time from infusion to REM was 17 min $\pm$ 9 after scopolamine pretreatment and 47 min $\pm$ 11 after saline ($p<.05$). These data are consistent with development of cholinergic supersensitivity following cholinergic blockade.

8. Identification of Stimulatory Cholinergic Mechanisms in Sleep-related Growth Hormone Secretion

We have examined the effects of cholinergic blockade with methscopolamine bromide, 0.5 mg intramuscularly, on sleep-related and insulin-induced growth hormone (GH) and prolactin (PRL) secretion. Thirteen normal young men were studied; eight had sleep studies, and at different times in 3 sleep subjects and in 5 others not studied during sleep, insulin tolerance tests were performed with 0.1 unit/kg insulin. After an adjustment night in the sleep laboratory, saline control and methscopolamine studies were done in a random sequence; study procedures included electroencephalographic, electromyographic, and electro-oculographic recordings, and blood sampling every 20 minutes for hormone radio-immunoassays. On methscopolamine nights, the mean $\pm$ SEM overall GH level of 2.89 + 0.44 and the mean peak GH level of 11.09 $\pm$ 3.11 ng/ml were dramatically reduced to 0.75 + 0.04 and 1.04 $\pm$ 0.25 ng/ml, respectively ($p<0.0001$ and $p<0.001$). Despite virtual absence of GH secretion during the night in every study subject, no measured sleep characteristic was significantly affected by methscopolamine, including percentage slow-wave sleep (12.1 $\pm$ 2.6% control vs. 10.3 $\pm$ 2.5% drug). Sleep PRL concentrations were not changed by methscopolamine. In contrast to the abolition of sleep-related GH secretion, administration of methscopolamine only marginally reduced the GH response to insulin hypoglycemia. Only one of ten time points differed significantly, and the peak concentrations, mean increments, and areas under the curves were not significantly different. We conclude that the burst of GH secretion which normally occurs following sleep onset is primed by a cholinergic mechanism which does not influence slow-wave sleep. Cholinergic mechanisms outside the central nervous system do not appear to play an important role in sleep-related prolactin secretion. The neurotransmitter mechanisms, and presumably the pathways, which subserve sleep-related GH secretion in man appear to be different from those which mediate the GH response to pharmacologic stimuli such as insulin.

9. Piperidine Enhances Sleep-related and Insulin-related Growth Hormone Secretion

Seven male normal adult volunteers slept in the laboratory, and blood samples were drawn every 20 minutes, after the lights were turned out at 11:00 p.m. A 30 minute IV infusion of piperidine or saline was started at sleep onset. In a comparison study, eight other volunteers had blood samples drawn for 2 hours after receiving 0.1 unit/kg regular insulin at 8:00 a.m. A 30 minute infusion of 100 mg piperidine or saline was started 15 minutes before insulin stimulation.

An analysis of variance revealed that piperidine significantly ($p<0.01$) enhanced sleep-related GH secretion over the 8 hours, from 4.22$\pm$0.46 ng/ml on saline to 6.09$\pm$0.78 ng/ml on piperidine ($p<0.02$ by paired t-test). This was particularly striking in the first two hours, when the values rose from 7.18$\pm$1.23 to 15.16$\pm$2.88 ng/ml ($p<0.02$). Similarly, there was enhancement of GH secretion during daytime insulin testing. The maximum GH increase after saline was 36.8$\pm$3.6 ng/ml compared to 48.0$\pm$4.3 ng/ml after piperidine ($p<0.01$). In order to determine whether the enhanced GH secretion was simply a summation of responses to insulin and piperidine separately,

we also infused piperidine 100 mg alone in seven volunteers. There was no effect on GH concentrations. Prolactin concentrations were also measured during the sleep and insulin studies; there was no significant effect of piperidine.

In sum, this provides further evidence of cholinergic mechanisms in the regulation of these forms of GH secretion, although the relative roles of nicotinic and muscarinic receptors are still unclear. There is little evidence that the cholinergic system plays a significant role in sleep-related or insulin-induced prolactin secretion.

10. Choline Chloride Reduces Insulin-induced Growth Hormone Secretion without Affecting Sleep-related Growth Hormone

Choline chloride (10 gm) or placebo was administered one hour prior to testing the effect of insulin-induced growth hormone release or prior to bedtime. Choline significantly reduced the insulin-induced growth hormone peak from 24 $\pm$ 4 to 17 $\pm$ 4 ng/ml ($p<.01$). No effect on nocturnal growth hormone or prolactin release was noted. The mechanism by which choline affected insulin-induced growth hormone is not clear, but probably does not result from increased cerebral acetylcholine synthesis.

B. Histamine: Effects of L-Histidine on Sleep

There is considerable indirect evidence suggesting that histamine may function in the brain as a neurohumor in sleep-waking mechanisms. To test the possible role of histamine in the control of waking in man, we administered its amino acid precursor, L-histidine, to human subjects and evaluated their electroencephalographic sleep patterns. L-histidine was given to three patients with intractable narcolepsy (20 gm per day for 2 weeks), to four normal volunteers (32.4 gm per day for 5 days) and to a patient with progressive systemic sclerosis (48.6 gm per day for 16 days). No effects were observed on nocturnal EEG sleep patterns in any of the subjects or on the symptoms of the narcoleptic patients. Although the degree to which histamine levels in the brain were elevated in this study is not clear, these results do not encourage the hypothesis that histamine is an alerting or waking factor in the brain.

C. Catecholamines

1. Differential Effects of D- and L-Amphetamine on the Sleep of Depressed Patients On and Off Lithium Treatment

Dextro (d-) and levo (l-) amphetamine produced different EEG sleep changes in seven depressed patients. Each patient received placebo or one of the isomers (30 mg base) at 7:25 a.m. in a double-blind fashion. The patients were studied under two conditions: without treatment with lithium carbonate and with treatment with lithium carbonate (0.9-2.1 gm/day beginning a minimum of 10 days before study). Both isomers reduced REM sleep and the proportion of total sleep spent in REM (the REM%). No REM rebound was observed on the night following REM suppression. Only d-amphetamine delayed sleep onset and reduced total sleep time, NREM sleep time, and sleep efficiency. The same changes were observed with and without lithium carbonate treatment.

2. Pimozide Attenuates d-Amphetamine-induced Sleep Changes in Man

Pretreatment with pimozide (mean dose=13 mg/day) blocked the effect of d-amphetamine (20 mg base, administered by intravenous bolus infusion at 0815) on all-night EEG sleep patterns in seven hospitalized psychiatric patients. Each patient was studied for five nights (2 nights baseline, 1 night on the day of the infusion, and 2 nights recovery) with and without pretreatment with pimozide. Without treatment with pimozide, d-amphetamine significantly reduced duration of total sleep, REM and nonREM sleep, Stage I, and Stage II. With coadministration of pimozide, d-amphetamine has no effect on sleep. These results suggest that the d-amphetamine-induced changes in sleep are mediated by dopaminergic neurons.

D. Serotonin:

1. Relationship to Narcolepsy

Since serotonin has been implicated in the induction and maintenance of sleep, we administered para-chlorophenylalanine (PCPA 3 gm/day for up to 4 weeks) to 3 patients with narcolepsy. No consistent change in sleep or clinical symptomatology was observed in the 3 patients as a group. One patient, in contrast to our previous experience with PCPA in patients with carcinoid syndrome, actually showed a significant increase in REM while on PCPA, while the other patients showed no decrease in REM. These results suggest (a) that narcolepsy is not related to an abnormality of serotonin and (b) that narcoleptics respond differently to PCPA than carcinoid patients.

2. Reduction of Brain Serotonin and REM Sleep by Acute Administration of a Tryptophan-free Amino Acid Diet

A variety of studies have suggested that the serotonergic system plays an important role in the regulation of sleep. In order to clarify this role, we have performed sleep studies on rats in whom brain serotonin was decreased by a new technique--the acute administration of a tryptophan-free amino acid diet.

Sleep studies were performed on five rats in a paired-data, crossover design. As can be seen on the Table, percentage REM sleep was decreased by one-third ($p<.05$), and there was a small but significant increase in nonREM sleep. Total sleep time was unchanged.

Our observation that an acute reduction in brain serotonin failed to produce decreases in nonREM sleep is in agreement with the results of two studies each in rats and humans. These data seem inconsistent with the hypothesis that the serotonergic system plays an important role in the production of nonREM sleep.

	Control Diet	Tryptophan-Free Diet	Statistical Significance
Total sleep	519.6±25.7*	529.8±27.0	NS
Sleep latency	45.5±10.6*	35.8±10.6	NS
Intermittent waking (IW/TRP)	33.9± 3.0	30.8± 2.2	NS
NonREM sleep (NR/TST)	87.6± 2.4	91.4± 2.3	$p<0.05$
REM sleep (REM/TST)	12.3± 2.4	8.5± 2.2	$p<0.05$

Values represent mean number of 30 sec. epochs ± SEM.

Effects of tryptophan-free diet on brain serotonin and 5HIAA

Hours after food presentation	Control diet		Tryptophan-free diet	
	Serotonin	5HIAA	Serotonin	5HIAA
2	1.43±0.04	0.52±0.06	0.80±0.02***	0.38±0.02**
4			0.62±0.01***	0.27±0.02***
8			0.44±0.02***	0.21±0.01***
24	1.52±0.02	0.56±0.04	0.65±0.02***	0.41±0.04*

Each value represents the mean from four rats + S.E.M., in units of ug/g brain tissue.

* $p<0.05$ by t-test when compared to mean of combined control values.
** $p<0.01$
*** $p<0.001$

These studies contribute to the present extensive national and international efforts to unravel the complex relationships of clinical disorders, biochemistry and sleep.

3. Intravenous Chlorimipramine Affects REM Cycle in Patients with Excessive Daytime Sleepiness and Control Subjects

The sleep of 5 normal volunteers and 4 patients with excessive daytime sleepiness was studied following intravenous administration of 5 mg chlorimipramine during the first period of rapid eye movement (REM) sleep. There was a 95% increase in the duration of the first REM cycle (time lapsed from onset of first REM period to the onset of the second REM period) in all 9 subjects, $p<.01$, compared to saline control nights. Other effects included a significant, $p<.05$, mean decrease in the post-infusion REM time and REM percentage to approximately one half of the control levels. Phasic events associated with REM sleep were also diminished during the post-infusion period with significant mean decreases in the REM density and the REM index per minute of sleep.

E. Ethanol:

1. A Dose-Response Study of the Acute Effects of Ethanol on the Sleep of Rats

We have administered increasing doses of ethanol (1.1, 1.5, 2.0, 2.5 g/kg) and equivolume saline intraperitoneally to five groups of six rats each. Injections were given five minutes before the beginning of seven-hour recordings, from 9:00 a.m. to 4:00 p.m. The percent of REM decreased and the percent of slow-wave sleep increased. This effect occurred most strongly in the first 3.5 hours of sleep. The number of awakenings were significantly increased in the second half of the recording. This may be a reflection of greatly decreased blood alcohol levels. (A dose of 2.5 g/kg is completely metabolized in about six hours in the rat.) It is of interest that even the highest doses of ethanol did not produce prolongation of total sleep time in either the whole recording or the first or second halves.

2. Hypnotics and Ethanol: Residual EEG Effects

The literature is divided between those who have found a possible synergistic interaction of ethanol and benzodiazepines and those who have not. We have examined this relationship by recording 12-minute waking clinical EEG's in 47 normal young men who received 0.25 mg triazolam, 30 mg flurazepam or placebo, alone or in combination with 0.8 g/kg ethanol. EEG's were performed as a baseline (3:00 p.m.), 50 minutes after receiving drug or drug placebo (5:50 p.m.), 50 minutes after receiving ethanol or ethanol placebo (7:05 p.m.) and 16 hours after having received the drug (9:00 a.m.). Criteria for the presence of drug effects were:

1. Increase in amount and amplitude of sinusoidal rhythmic beta activity.
2. Lower amplitude of background activity.
3. Decrease in amount of alpha activity.
4. Presence of "well-modulated" alpha.
5. Presence of "drug spindles."

Eighty-five percent of the baseline records were correctly identified as having no drug effect. Analysis of the second EEG (50 minutes after drug) and third EEG (50 minutes after ethanol) showed that our interpretations had a significant relationship to whether or not the subject had received the active drug ($p<0.02$ and $p<0.05$, respectively). These findings lead us to believe that this method for determining the presence of drug effects is valid.

Analysis of the fourth EEG--16 hours after drug administration--showed no overall difference in incidence of EEG drug effects among the various combinations of drugs and ethanol. Neither drug alone was different from placebo, nor was either drug alone different from itself plus ethanol.

Flurazepam plus alcohol produced drug effects in 86% of the records, compared to 22% in the placebo group ($p<0.019$; Fisher Exact Probability Test). The difference between incidence of drug effects following

triazolam plus ethanol (56%) and placebo (22%) did not reach statistical significance. There was no difference in frequency of effects between flurazepam plus ethanol and triazolam plus ethanol.

These data lead us to believe that ethanol produces a small but significant increase in the duration of EEG effects of flurazepam. Flurazepam alone is known to remain active for some time (the major metabolite may have a half-life of 50-100 hours). Thus, repeated use of the drug might produce cumulative effects which may possibly be increased by ethanol.

3. Sleep, Behavior, and Blood Ethanol Concentration in the Ethanol-Dependent Rat

In this study sleep recordings were performed to further characterize a previously-described animal model of ethanol dependence. In summary, this consists of administering approximately 10 gm/kg of ethanol per day by intragastric intubation in six divided doses for four days. Eight-hour EEG recordings were made during the baseline period and the first and third days of ethanol administration (induction period). One group of animals (N=14) was also recorded on the first and fourth withdrawal days; a second group (N=5) was recorded on days 1-4 and 6 of withdrawal. During the induction period, REM sleep time was decreased, particularly on the third ethanol day when blood ethanol concentrations were highest.

This largely reflected decreases in the number of REM sleep episodes. The first day of ethanol withdrawal was associated with a decrease in total sleep time, primarily due to loss of nonREM sleep. Total sleep time gradually returned to normal, and in fact slightly exceeded baseline levels on the third withdrawal day. At this time, REM percentage increased to twice the baseline values. This was related to a decrease in REM latency and an increase in the number of REM sleep episodes. REM percentage then decreased such that on the fourth withdrawal day it approached baseline values. Total sleep time and other sleep parameters were also indistinguishable from baseline on the fourth day. Blood ethanol concentrations on the first withdrawal day were inversely related to behavioral scores of withdrawal and to EEG sleep latency.

The major observations in this model seem consistent with observations in the literature on ethanol withdrawal in animals and humans. Several of the EEG parameters--notably the initial decreased sleep and later REM rebound-- may be useful in testing new treatments for ethanol dependence.

F. Enzymes: The Relation of Platelet MAO to Human Sleep

We have examined the sleep of normal volunteers with high and low platelet MAO activity. These volunteers were drawn from groups with MAO activity in the highest and lowest decile of a population of 375 students. Ten high ((mean 16.1±3.2 (sd) nanomoles per 10^8 platelets per hour)) and ten low ((7.1±2.3 (sd)) volunteers, 5 men and 5 women in each group returned for sleep studies. Each subject was recorded for two nights following two adjustment nights.

The group with high platelet MAO activity had significantly lower REM sleep time (69.9+5.2 vs 92.7+8.6 minutes; $p<0.05$), lower REM density 1.5+ 0.1 vs 1.9+0.1, and presumably as a result of the previous two factors, a lower REM index, a measure of the total number of eye movements (105.5+ 9.3 vs 184.8+21.8; $p<0.01$). All other sleep variables were similar for the two groups. These observations constitute the first report relating individual differences in an enzyme involved in neurotransmitter metabolism to human sleep EEG parameters.

G. Arginine Vasotocin Alters REM Sleep in the Rat

Arginine vasotocin (AVT), a pineal peptide, has been reported to inhibit gonadotropin release, modify conditioned behavior, and enhance slow-wave sleep. The latter effect was particularly striking because of the remarkably small doses employed--10^{-6} picograms intraventricularly and 1 microgram intraperitoneally in the cat.[1]. In order to further characterize the effects of this substance, we are reporting here a dose-response study on sleep and behavior in the rat.

Arginine (8) vasotocin was given intraperitoneally to 200 gm male Sprague Dawley rats in doses of 0.5 ug/kg (n=9), 50 ug/kg (n=9) and 500 ug/kg (n=5), and the EEG and EMG were monitored for 8 hours starting at 8:30 a.m. In a second study, 10 rats received 10 ug/kg at 8:30 a.m. and the following behavioral measures were done: righting reflex, an equilibrium test, grasping reflex, catatonia, and tail pinch reflex.

The lowest dose (0.5 ug/kg) had no effect on the sleep stages. The middle dose (50 ug/kg) decreased REM latency by 65 percent to about 29 minutes ($p<0.02$) and decreased ($p<0.008$) REM efficiency from 93.3+ 1.0 (saline) to 86.9+2.0 (AVT). There was no effect on sleep latency or total sleep. When data were broken down into two hour periods, the major change was a 55% decrease in REM time ($p<0.02$) in the second two hours. During the first two hours of recording, the animals, when awake, assumed a characteristic quiescent crouching posture, and seemed less responsive to mildly disturbing stimuli such as air blown in the ear. The systematic behavioral tests did not reveal significant changes, although there was a trend toward remaining longer on the equilibrium bar.

At the highest dose (500 ug/kg) four out of the five rats died of pulmonary congestion and edema within a few hours.

These data indicate that 50 ug/kg has potent physiological effects. Although we did not confirm a report of increased slow-wave sleep in cats[1], our REM sleep data are reminiscent of the report of decreased REM in the first 5 hours. This also seems to confirm the observation that animals sleeping after AVT administration are easily aroused; when awake they appeared quiescent, yet did not have the neurologic deficits often associated with sedative/hypnotic agents.

H. The Effects of GABA Agonists and Antagonists on Sleep and Behavior in the Rat

It has been estimated that gamma-aminobutyric acid (GABA) functions as a neurotransmitter in 30 percent of mammalian synapses. Given its ubiquitous nature, and recent evidence that some of the effects of benzodiazepine anxiolytics and hypnotics (e.g., the anticonvulsant properties) may be mediated by GABA; it seemed appropriate to carefully assess the effects of GABA agonists and antagonists on EEG measures of sleep. We are reporting the results of administration of 2 and 4 mg/kg of picrotoxin (a GABA antagonist), 82 and 164 mg/kg of imidazole-4-acetic acid or IMA (a GABA agonist) and saline on the sleep and behavior of the rat. All drugs were given at 8:30 a.m. intraperitoneally to 175 gm male Sprague-Dawley rats (approximately 9 in each group), and recordings were performed for 8 hours; because of the relatively short half-lives of these agents, data were also broken down into two-hour segments.

Analysis of the recordings revealed prominent seizure activity in three animals who received the high dose of picrotoxin; they were excluded from the sleep data. An ANOVA demonstrated that among the remaining animals there were significant differences in total sleep ($p<0.02$) and nonREM sleep ($p<0.02$) during the first two hours. A Neuman-Keuls analysis revealed that the high dose of IMA increased total sleep to 60.4 ± 8.5 min compared to 23.3 ± 4.0 min for the high dose of picrotoxin ($p<0.01$). Saline control values for total sleep primarily represented alterations of nonREM sleep.

The effects of these agents on several behavioral tests were assessed in 7 rats in each group. These measures included tail pinch, righting reflex, coordination on a balance bar and catalepsy (time remaining motionless with front feet placed on a horizontal bar). Of these, only the catalepsy test approached significance ($p<0.06$), indicating prolonged times with the high dose of IMA.

These data seem to confirm previous work suggesting that GABA agonists have sedative qualities. Further studies will be needed to assess the possible role of GABA in natural and pharmacologically-induced sleep.

I. Studies with the Delta Sleep-Inducing Peptide in the Rat.

As part of a series of studies on possible circulating sleep factors, we have examined the effects of a "Sleep Peptide" marketed by Calbiochem (No. 567295), with an amino acid sequence identical to the "delta-sleep-inducing peptide." Originally this material had been discovered in the CSF of rabbits undergoing thalamic stimulation, and was reported to induce delta activity and spindles when administered to rabbits. It was later synthesized and reported to have similar actions. In the studies reported here, the Sleep Peptide or artificial CSF (control) was administered intraventricularly to 200 gm male Sprague-Dawley rats. Drug administration was at 8:30 a.m., under conditions in which lights were on daily from 8:00 a.m. to 8:00 p.m. Recordings lasted 8 hours, and were interpreted in 30 second epochs as waking, nonREM and REM sleep.

Rats were studied with doses of 5.1 ug/kg (n=11) and 25.5 ug/kg (n=10). When data from the total 8 hour recording with the lower dose were examined, the only significant change was an increase in sleep latency (18.7+4.0 with control injection to 32.7+ 5.5 min with active drug; $p<0.009$) and a decrease in nonREM sleep (282.0±13.7 to 249.7±17.6 min; $p<0.04$). When the first two hours alone were considered, the only significant change was a slight decrease in the number of awakenings (6.4±0.5 to 5.1±0.7 min; $p<0.05$). At the higher dose, the only significant change was an increase in intermittent waking time (108.4±8.0 to 128.4±7.0 min; $p<.039$) over 8 hours, and an increase in REM-nonREM cycle length (14.0± 6. to 27.2±5.4 min; $p<0.02$) in the first 2 hours.

In addition to the sleep studies, a pilot study of the effect of the same 2 doses of the Sleep Peptide on the motor activity of 13 rats was conducted. No gross changes in motor activity were noted.

In summary, intraventricular administration of the Sleep Peptide to rats was found to have only minor effects on the sleep stages, and if anything they were in the direction of slightly disturbed sleep.

J. Lifetime Inhibition of Monoamine Oxidase: Effects on sleep in the rat.

Monoamine oxidase (MAO) inhibitors have been widely recognized to be potent suppressors of REM sleep in humans, although most studies have involved inhibitors of both MAO Type A and B, and have been only a few weeks in duration. In this study we have examined the effects of clorgyline, a Type A inhibitor, on the sleep of the rat, both when given subacutely and when given over the lifetime of the animal. In the subacute study, clorgyline 2 mg/kg was given daily for 3 days, and an 8 hour sleep recording was performed shortly after the final injection. Biochemical analysis confirmed that MAO-A was significantly ($p<0.0001$) inhibited by over 90%. It was found that in the 12 rats receiving clorgyline REM sleep time was decreased to 21.5 + 3.0 min. compared to 33.6 + 2.7 min. for the 10 control animals ($p<0.01$). The REM-nonREM cycle length was increased to 39.0 + 6.2 min. after clorgyline compared to 29.2 ± 2.2 min. for controls ($p<0.05$).

In the second experiment pregnant rats were implanted with osmotic pumps releasing 1 mg/kg of clorgyline per day, and after birth and weaning the pumps were implanted in the new generation to give a similar dose. When these rats matured, sleep EEG and biochemical studies were performed. Biochemical analysis again revealed that MAO-A was inhibited by over 90% ($p<0.001$) while MAO-B was unaffected. It was found that despite these marked changes in MAO-A activity the sleep of the 7 clorgyline rats was similar to that of the 7 controls. This may suggest that during chronic MAO inhibition compensatory mechanisms, possibly including altered receptor sensitivity, allow sleep to return to normal.

K. Where is the Hypnotoxin?

It has been hypothesized for many years that a physiologic circulating substance induces sleep and waking behavior, and a number of possible substances have been proposed. In the past no single laboratory has compared

these various materials. For this reason we have examined the effects on EEG-defined sleep in rats of a substance modeled after that of Nagasaki et al.[1], the delta sleep-inducing peptide (DSIP), Factor S derived from the brains of sleep-deprived rabbits, and arginine vasotocin (AVT). Data on the delta sleep-inducing peptide and arginine vasotocin have been presented earlier[2,3]. Intraventricular administration of 5.1 and 25.5 ug/kg of DSIP was without effect on sleep. Intraperitoneal administration of 50 ug/kg of AVT reduced REM latency and efficiency but did not affect total sleep time or sleep latency. (A lower dose, 0.5 ug/kg was without effect and 500 ug/kg was lethal.) Material from the brains of rats sleep-deprived for 24 hrs.[4] was derived along the general methods described by Nagasaki et al.[1] The amount derived from the brains of 6 sleep deprived rats was given to each of 10 rats (active group) and that derived from 6 normal rats was given to each of 7 control rats. Other controls included saline (N=10), material which had been boiled (N=9) and material exposed to a protease (N=9). After injections at 8:30 a.m., the only major significant changes were an increase in REM efficiency and decrease in the number of waking episodes during the first 4 hours, but even in these cases the boiled and protease-treated material produced effects similar to the "active" material. A study of intraventricular administration seemed also to suggest very little effectiveness. In another study, Factor S obtained from Dr. John Pappenheimer was given intraventricularly over 30 min. starting 8:00 p.m. The dose of Factor S was the amount of material derived from 33 gm of brain tissue. It was found that Factor S significantly prolonged sleep latency (6.2 $\pm$ 3.0 min. for control, 46.1 $\pm$ 12.1 min. for Factor S; $p<0.007$), although once asleep there was a reduction in intermittent waking time during the first two hours (66.0 $\pm$ 4.0 min. for control, 40.3 $\pm$ 6.1 min. for Factor S, $p<0.01$). Total sleep time was unaffected. Taken together, these data would seem to call for a careful re-evaluation of the existence of currently hypothesized physiologic sleep-promoting substances.

L. Failure of an Inhibitor of Dimethyltryptamine Synthesis to Affect Sleep in Rabbits.

It has been speculated for some time that the conversion of indoles to methylated indoles such as dimethyltryptamine (DMT) may play a role in the genesis of psychotic illnesses. Because of longstanding interest in possible sleep regulating mechanisms involving indoleamines, and reports of altered sleep patterns in schizophrenia, we have examined the effects on sleep of N,N'-Bis- (3-methyl-2-thiazolidinylidene) succinamide, an inhibitor of indoleamine-N- methyltransferase. We gave 68 mg/kg of active drug and saline by acute oral administration at 8:30 a.m. to 9 young male rabbits, and recorded sleep stages for 8 hours. It was found that the agent had no statistically significant effects on sleep.

M. Effects of a Brain Extract from Sleep-deprived Rats on Benzodiazepine Receptor Binding.

The presence in brain of stereospecific binding sites for benzodiazepines raises the possibility that these receptors mediate the therapeutic actions of this important class of sedative/hypnotics. The receptor-binding affinity of various benzodiazepines appears to parallel clinical anxiolytic dosage, potency in the rat conflict test and ability to block metrazol(R)induced seizures; the relation to the sleep-inducing

properties of these agents is not yet known. In order to further examine this relationship we have examined the effects of extracts from the brains of sleep-deprived rats on receptor binding in vitro. In each of a series of 5 experiments, 6 rats deprived of sleep for 24 hours and 6 control rats who were allowed to sleep ad lib were killed, and the brains were quickly removed and frozen. The tissue was homogenized in ice-cold buffer (PH 7.4 Tris HCl), and the homogenate was centrifuged at 100,000 g for 60 min. under nitrogen pressure. The resulting supernatant was concentrated with an Amicon PM-10 filter (exclusion limit > 10,000 daltons), and the filtrate was lyophyllized and re-constituted in distilled water. Addition of progressively larger amounts of the diazepam crude extract from control rats produced significantly more inhibition of specific H^3-diazepam binding compared to extract prepared from sleep-deprived rats in four out of five studies. These data suggest that a substance from the brains of sleep-deprived rats may either enhance binding (or reduce inhibition of binding) of benzodiazepines compared to a comparable extract from controls.

N. Local Cerebral Metabolic Activity in Wakefulness and NREM Sleep

Local cerebral glucose utilization has been examined in 74 brain regions with the 2-deoxyglucose method. Mean glucose utilization was lower during NREM in all areas compared with wakefulness. Selected regions are shown (statistically significant in 20 regions) in Table. Units are nmol/100 gm/min.

	Awake	NREM	% Change	p
Striate cortex	52 ± 9	34 ± 8	-35	.054
Inf. parietal cortex	45 ± 5	29 ± 3	-35	.009
Auditory cortex	82 ± 4	52 ± 11	-30	.014
Med. orbital cortex	53 ± 9	33 ± 3	-36	.02
Lat. genic. body	41 ± 7	25 ± 2	-39	.018
Med. genic. body	73 ± 6	47 ± 3	-57	.003
Dorsal med. nuc., thalamus	52 ± 7	34 ± 4	-36	.017
Reticular nuc., thalamus	29 ± 7	17 ± 2	-40	.05
Pulvinar	43 ± 3	30 ± 4	-31	.006
Supra optic nuc.	29 ± 2	23 ± 3	-23	.05
Med. septal nuc.	28 ± 2	21 ± 2	-32	.02
Red nucleus	52 ± 5	37 ± 2	-29	.009
Mes. reticular	33 ± 2	22 ± 3	-35	.003
Dorsal raphe	36 ± 5	30 ± 4	-18	.14
Ventral raphe	36 ± 9	29 ± 3	-18	.28
Sup. colliculus	55 ± 6	38 ± 4	-32	.01
Flocculus	53 ± 8	29 ± 4	-46	.008
Locus ceruleus	34 ± 6	29 ± 4	-16	.25
Corona radiata	8 ± 1	4 ± 0.1	-50	.09

The results are consistent with recent studies showing decreased cerebral blood flow during NREM sleep. No metabolically active (NREM) sleep "center" was found.

II. Hormones:

A. Regulation of Sleep-Related Growth Hormone and Prolactin Secretion

The study of pharmacologic manipulation of sleep-related growth hormone (GH) and prolactin (PRL) secretion has provided data on the roles of the serotonergic, noradrenergic and cholinergic systems in anterior pituitary regulation. The serotonin receptor blocker methysergide (2 mg PO q 6 h) was administered for 48 hours to normal young adults, and blood samples were drawn every 20 minutes during eight-hour sleep recordings. Methysergide administration was associated with an increase in several measures of sleep-related GH secretion. Mean peak concentrations of GH rose from 7.86 ± 1.35 (SEM) ng/ml on control nights to 10.30 ± 1.39 ng/ml with methysergide ($p<0.03$). Mean nocturnal PRL concentrations were decreased by 70.3%. In contrast to the increases in sleep-related GH secretion, daytime insulin-stimulated GH secretion was decreased 36.4% by methysergide.

These studies would seem to imply that: (1) serotonergic mechanisms may have an inhibitory role, and cholinergic mechanisms may have a facilitory role, in regulation of sleep-related GH secretion; and (2) data drawn from pharmacologic manipulation of daytime insulin provocative testing may lead to erroneous conclusions regarding physiologic control of GH secretion.

B. The Effect of Dexamethasone on Sleep-Related Growth Hormone Secretion in Congenital Adrenal Hyperplasia: Therapeutic Implications

Treatment of children with 21-hydroxylase deficiency congenital adrenal hyperplasia (CAH) with steroids has two goals: replacement of inadequate secretion of cortisol (and sometimes aldosterone) and suppression of oversecretion of adrenal androgens. In adults, and adolescents who have achieved full growth, it is most desirable to use long-acting steroids such as dexamethasone (DEX), given at midnight. The effects of DEX on nocturnal GH secretion need to be evaluated, however, particularly before use in children and adolescents who have not achieved full stature. For this reason we have examined GH secretion and other endocrine measures during sleep in CAH patients receiving DEX in two different regimens.

The four females and two males who participated in the study ranged in age from 13 to 22. All but two (who were female) were salt losers. All patients received 0.5 mg DEX for six days each at 0700 and 2300 hours. Sleep studies were performed on night 5, and during a study in which several blood samples were drawn on night 6.

Examination of 24 hour excretion of 17 ketosteroids and pregenanetriol showed adequate suppression under both regimens in four of the six patients. In two subjects there was inadequate suppression with either regimen. LH was found to be secreted episodically throughout the night in a similar and normal manner with both regimens. Mean nocturnal FSH concentrations were also similar.

Examination of sleep-related GH revealed increased secretion during the first two hours of sleep when DEX was given at 0700 compared with administration at 2300. The areas under the curves of GH plots during this

time were 1737+744 (SEM) and 666+359 ng/ml-min respectively ($p<0.05$). Following administration at 2300, there was a tendency for increased GH secretion later at night, which did not, however, reach significance.

Only two significant differences were noted on sleep EEG recordings made during the blood study: Following 0700 administration, there was a longer sleep latency (65.8±18.1 vs 24.2±7.9 minutes; $p<0.02$) and decreased nonREM sleep (266.8±34.4 vs 363.3±13.7 minutes; $p<0.02$). These observations were not borne out by the recording on night 5, and hence are of questionable biological importance. The possibility remains, of course, that DEX affects sleep differently when patients undergo the slight stress of an intravenous study compared to when they do not.

These observations suggest that administration of DEX at 0700 suppresses the adrenals yet permits sleep-related secretion of GH. Further studies of chronic use, and considerations of possible effects on somatomedins, will be necessary to further establish the safe use of DEX in children and adolescents.

C. LH-RH Administration and Human Sleep.

Luteinizing hormone-releasing hormone (LH-RH), a decapeptide of hypothalamic origin, stimulates release of LH and FSH from the pituitary; in addition it may possess direct actions on the central nervous system. It has been shown, for instance, to cause mating behavior in estrogen-primed ovariectomized rats. Its administration has been reported to produce sexual arousal in human males with hypogonadism well before androgen levels reach the normal range. Because of these implications that LH-RH may possess central effects, and since gonadal hormones have been reported to influence sleep, we have explored the possibility that LH-RH administration may influence normal human sleep.

Subjects were 5 male and 5 female young adult normal volunteers. Females were studied during the follicular phase of the menstrual cycle. Following an adjustment night, each had sleep studies on two nights. On one night they received 100 mg of LH-RH in 500 cc of normal saline intravenously over an 8 hour period starting when the lights were turned out at 11:00 p.m. On the other randomly-sequenced night they received an equivalent volume of normal saline. The dose of LH-RH, while lower than that reported to produce human sexual arousal, has been shown to acutely raise serum LH and FSH.

Sleep EEG data revealed that LH-RH administration produced no change in the total minutes or percentage of the sleep stages, total sleep, or sleep latency. The subjects were also divided into two groups by sex, and once again there were no significant changes in sleep. It is concluded that low doses of LH-RH, sufficient to stimulate gonadal hormones, have no acute effects on normal human sleep. The possibility remains, of course, that higher doses or more potent analogues of LH-RH may have such actions.

D. Effects of Melatonin and Propranolol on Sleep in Rats

Melatonin and L-propranolol, which inhibit melatonin synthesis, were administered to rats at 07.45 h and 19.45 h. Melatonin given in the morning decreased nonREM sleep, but when given at night had no effect on sleep stages. L-propranolol given in the morning had no effect on nonREM sleep, but increased it at night. L-propranolol produced decreases in percentage REM sleep at both times.

E. Changes in Human Sleep Induced by Growth Hormone Administration

Although it has been known for some time that growth hormone (GH) is secreted in relation to sleep, the physiological significance of this phenomenon is unclear. It has been reported in animals that intraperitoneal injections of GH rapidly enter the brain and alter metabolism of biogenic amines, compounds which may play a role in processes such as thermoregulation, memory and sleep. We are reporting here the effects of GH administration on sleep, mood and memory in humans.

Eighteen normal young adult volunteers (16M, 2F) received IM injections of GH and saline 15 minutes before bedtime at 11:00 p.m. and sleep EEG, EOG and EMG were recorded for 8 hours. Doses of GH were 2 (n=8) and 5 (n=10) international units. In a companion study 15 subjects received injections of 2 or 5 units GH at 8:00 a.m., and had the following measures: a mood and behavior scale, 100 mm line measures of alertness and anxiety, serial learning lists, and the Buschke selective reminding test.

The lower dose of GH had no effects on sleep. The higher dose induced a 19% decrease in slow-wave sleep ($p<0.01$) and a 13% increase in REM sleep ($p<0.05$). In order to get a sense of GH concentrations related to these changes, serial blood sampling was done in two patients receiving 5 units. It was found that the injections resulted in peak concentrations of 60-80 ng/ml, dropping to less than 10 ng/ml by 7:00 a.m. Other measures, including sleep latency and total sleep, were unaffected. Neither dose, when given in the daytime, affected the mood and memory tests.

This study confirms previous work in animals, indicating that GH administration induces an increase in REM sleep, and also describes a decrease in slow-wave sleep. It raises the possibility that GH secretion may play a role in sleep-stage regulation.

F. Plasma Melatonin Concentrations During Human Sleep

In order to further define the relation of plasma melatonin to sleep, we have studied samples from six normal young adult volunteers (3M, 3F) and analyzed melatonin by means of a new, more sensitive mass spectrometric assay. Samples were drawn every 20 minutes from an indwelling venous catheter from 11:00 p.m. until 7:00 a.m., which coincided with the hours of darkness.

An analysis of variance revealed significant differences between subjects ($p<0.0001$), different times of the night ($p<0.0001$) and state of consciousness ($p<0.009$). Concentrations rose during each of the 3 sequential time periods, progressing from 22.3+4.4 u g/ml in hours 1 and 2, to 38.4±2.8 in hours 3 and 4, to 56.6+2.9 in hours 5 to 8. Analysis of the states of consciousness revealed that samples drawn during intermittent wakefulness were significantly ($p<0.01$) lower than during the rest of the sleep stages combined, and showed no significant relation to the individual stages.

Previous studies, using radioimmunoassays have indicated either a mean increase at roughly 2:00 a.m. or apparently random peak times; the present work seems to indicate gradual increasing concentrations of melatonin throughout the night. One report indicated no consistent relationship to individual stages of sleep, a finding borne out here. The significantly decreased concentrations during wakefulness, however, suggest that sleep, along with darkness and clock time, may influence melatonin secretion.

G. Effects of Subacute Administration of Growth Hormone on Sleep-related Growth Hormone Secretion.

Previous studies have reported that following repeated administration of growth hormone (GH) there is decreased endogenous GH release in response to insulin[1]. This has been taken to suggest the presence of a negative feedback regulatory mechanism, at least with regard to GH secretion in response to relatively unphysiologically low blood glucose concentrations. We have examined whether repeated injections of GH will diminish the more physiologic sleep-related release of GH. Six young adult normal volunteers were given 2 units of human GH and equivolume saline intramuscularly every 12 hours for a total of 5 injections in a paired-data design study. The final injection was at 5:00 p.m. on the night of the sleep study, so that by bedtime little exogenous GH was circulating, although somatomedin activity was presumably elevated. Sleep EEG data indicated that this procedure had little effect on traditional sleep parameters, most notably no change in delta sleep. On the other hand, an analysis of variance revealed a significant ($p<0.0001$) effect on the overall night's GH secretion, such that mean plasma concentrations dropped from 4.6 + 0.7 ng/ml following saline to 2.4 ± 0.2 ng/ml after exogenous GH. There was a significant ($p<0.0003$) treatment-time interaction, indicating that the decrease was most evident in the first two hours of sleep (10.1 + 2.3 ng/ml after saline vs. 3.8 ± 0.40 ng/ml after GH). These data seem to suggest that the nervous system is sensitive to the effects of previously high concentrations of circulating GH, and may respond by decreasing sleep-related GH secretion.

H. Effects of Calcitonin on Human Sleep

A previous study[1] has suggested that administration of synthetic salmon calcitonin has daytime tranquilizing or depressant effects in manic or other psychotically agitated patients, yet may reduce nocturnal sleep as determined by 30 minute nurses' sleep checks. This led us to examine the effects of calcitonin on human sleep. Seven young adult normal volunteers

were given skin tests to determine absence of allergy, then were administered 140 MRC units of calcitonin (Calcimar^{R}) subcutaneously shortly before bedtime. Two subjects subsequently experienced nausea, and their data is not included in this study. Analysis of polygraphic sleep data revealed relatively minor changes in sleep, including an increase in stage 1 (15.2 + 3.2 min. with saline vs. 28.3 $\pm$ 4.2 min. with calcitonin, $p<0.05$) and a non-significant increase in intermittent waking time (9.4 $\pm$ 6.3 vs. 22.7 $\pm$ 3.2 min., $p<0.06$). This seems to suggest that calcitonin may have minor effects on polygraphically-defined sleep, leading perhaps to mild disturbance.

III. Major Findings: Clinical Disorders

A. Successful Separation of Depressed, Normal, and Insomniac Subjects by EEG Sleep Data

Data from all-night EEG sleep studies were used to distinguish normal subjects, primary depressed patients, and primary insomniac patients. In part 1, we compared 41 normal subjects, 56 depressed patients, and 18 insomniacs. In a univariate comparison with normal subjects, depressed patients showed less total sleep, longer sleep latency, more early morning awake time, more intermittent awake time, less delta sleep, less sleep efficiency, and shorter rapid eye movement (REM) latencies; compared with insomniacs, depressed patients showed greater early morning awake time, shorter REM latency, greater REM index, and greater REM density. Using multivariate discriminant analysis, 82% of the sample were correctly classified by diagnosis: 100% of the normal subjects, 72% of the depressed patients, and 77% of the insomniacs. Eight variables contributed to the multivariate separation of depressed individuals from insomniacs and normals: total sleep time, total recording period, sleep efficiency, sleep latency, early morning awake time, awake time, REM time, and REM %. When the discriminant functions were applied to a second group of 18 primary depressed patients, 82% were correctly classified as depressed. These results suggest that primary depressed patients and primary insomniac patients may show relatively characteristic patterns of sleep abnormality.

Part I: Classification of Normal Subjects, Depressed Patients, and Insomniac Patients by Discriminant Analysis: All 12 Sleep Variables

		No. (%)	
	Normal	Depressed	Insomniacs
Normal (N=41)	41 (100)	0 (0)	0 (0)
Depressed (N=56)	13 (23.2)	41 (73.2)	2 (3.6)
Insomniac (N18)	4 (22.2)	0 (0)	14 (77.8)

B. The "Switch Process" in Manic-depressive Illness: Circadian Variation in Frequency of Switches and Changes in Total Sleep Time.

A "switch" was operationally defined as a rapid change in mood (change of three or more points on a 15-point scale) occurring in less than 24 hours.

Out of 75 bipolar manic-depressive patients, 35 showed 70 switches from depression to mania (D→M) and 37 switches from mania to depression (M→D). Both D→M and M→D switches occur predominantly in the morning (7 a.m.- 3 p.m.). Among the D→M group, patients who switch at night (11 p.m.- 7 a.m.) show significantly greater sleep loss for the next four days (Day 0 to +4), than if they switch in the morning (7 a.m. - 3 p.m.) ($p<0.01$, Newmann Keuls test). The evening shift does not differ from the other times. The M→D switches, however, fail to show a similar differentiation between the three time shifts with regard to total sleep time. These data indicate that time of switch may predict subsequent sleep changes in D→M switches.

In an unmedicated female bipolar patient fifty-eight all-night polygraphic sleep recordings were obtained during eight switches into and out of mania. While depressed she was hypersomniac and exhibited elevated REM sleep time and short REM latency. On four nights, she apparently switched into mania while asleep. The last recorded sleep stage in each case was REM sleep.

We have analyzed 144 switches (80 switches into mania and 64 out of mania) in another bipolar patient with regular 48-hour manic-depressive cycles. Switches into mania showed a sharp tendency to occur between 8 p.m. and 8 a.m. with peak incidence between 2 a.m. and 4 a.m. Similarly, switches out of mania occurred significantly more often between 4 a.m. and 6 a.m. than during other time periods. The frequency of distribution of these switches into and out of mania was significantly different from an expected random distribution (2XR chi square analysis with df=11). Switches during sleep: among the 80 switches into mania 37 occurred out of sleep (41%); among the 64 switches out of mania, 27 were associated with sleep (42%).

C. The Relationship between Changes in REM Sleep and Clinical Improvement in Depressed Patients Treated with Amitriptyline

EEG sleep recordings were obtained on consecutive nights from six hospitalized depressed patients before, during, and after treatment with amitriptyline. Amitriptyline significantly reduced time spent in Rapid Eye Movement (REM) Sleep and prolonged the REM Latency throughout the treatment period. Three patients who improved during treatment had a REM rebound when amitriptyline was discontinued, whereas three patients who did not improve showed no REM rebound.

D. Muscarinic Supersensitivity: A Possible Model for the Sleep Disturbance of Primary Depression

The sleep changes induced in eleven normal volunteers following the administration of scopolamine on 3 consecutive mornings resembles many of the abnormalities observed in the sleep of patients with primary depression: increased sleep latency, and reduced REM latency, total sleep time, and sleep efficiency. Furthermore, multivariate discriminant analysis, previously shown to distinguish the sleep records of depressed patients from those of normal controls and insomniac patients, selected the records from baseline nights as normal and those after scopolamine as predominantly depressed.

	Baseline	Scopolamine
Normal	11	4
Depressed or Insomniac	0	7*

$p < .005$ Fishers Exact Probability Test

* depressed 6
insomniac 1

These observations suggest that muscarinic supersensitivity in normals may function as a pharmacological model for the sleep disturbances of depression.

E. Faster Cholinergic REM Induction in Remitted Patients with Primary Affective Illness

We compared remitted patients with primary affective illness and normal control subjects on the "cholinergic REM-induction test." In this test we measure the speed with which REM sleep is induced by arecoline, a cholinergic muscarinic agonist administered intravenously during the second nonREM sleep period.

The control subjects were sixteen paid normal volunteers (9 males, 7 females, mean ± SD, age = 28.3 + 5.4 years). They were compared with two groups of remitted patients with primary affective illness. The initial group (Group I) of thirteen remitted patients (4 males, 9 females, 12 bipolar and 1 unipolar, mean age 28.9 ± 6.9 years) was tested after all their regular medications had been discontinued for at least 2 weeks. To rule out potential confounding effects of prior psychoactive drug treatment or withdrawal on the arecoline response of Group I, we recruited a second group (Group II) of eight remitted patients who had never received any somatic therapy (4 patients) or had not received it within 4 months of the study (4 patients). The second group consisted of 6 bipolar and 2 unipolar affective patients (3 males, 5 females, mean ± SD, age = 29.1 ± 5.6 years). All patients met research diagnostic criteria for primary affective illness and had been in clinical remission for at least 3 months at the time of the study.

Arecoline induced REM sleep significantly faster in both patient groups than in controls. Following arecoline 0.5 mg, mean Inf-REM_2 latency was 38 min in normals, 11 min in Group I, and 15 min in Group II ($p<.01$, $H=10.22$, $df=2$, Kruskal-Wallis one-way analysis of variance). The mean Inf-REM_2 duration was significantly shorter for both Group I ($p<.01$) and II ($p<.025$, Mann-Whitney U-test) than for normal controls. Arecoline 1.0 mg was administered only to normal controls and Group I; again, the patients entered REM significantly faster than controls (7 min versus 9 min, $p<.01$, Mann-Whitney U-test).

G. Catecholamine Depletion: Sleep and Mood Changes in Man Mimic the Bipolar Switch Process

Alpha-methyl-para-tyrosine (AMPT, 3 gm/d) was administered in a double-blind fashion to 9 normal volunteers (mean age 24) for 3 days. During AMPT administration, such ratings of mood indicated significantly increased depression, while sleep studies indicated hypersomnia and reduced REM sleep. During withdrawal from AMPT mood ratings suggested hypomania and sleep studies indicated a transient hyposomnia. Co-administration of lithium (900-1200 mg/day) attenuated the withdrawal hypomania. These results suggest that AMPT in normals mimics the bipolar "switch" process from depression to mania.

H. Sleep of Bipolar and Unipolar Depressed Patients Compared

The sleep of 36 normals, 36 unipolar patients, and 22 bipolar patients was compared. The only sleep variable which significantly differed between unipolar and bipolar patients was REM efficiency. Bipolar patients showed a significant reduction compared with unipolar patients (82% $\pm$ 3 versus 91% $\pm$ 1, $p<.001$). In addition, "hypersomnia" (defined as more sleep than the age-corrected normal mean) was more prevalent among the bipolars than the unipolars ($p<.05$, $X^2 = 3.81$).

I. Schizophrenia

1. Schizophrenia:

Eight actively ill schizophrenics and eight nonpsychotic controls were deprived of REM sleep by the awakening method for two nights. Sleep patterns during five post-deprivation nights were analyzed by a variety of multivariate techniques.

Data suggest that actively ill schizophrenics are less likely than control psychiatric patients to exhibit a normal REM rebound. They require fewer awakenings than controls to achieve REM deprivation. They show little or no change in REM time or REM% during recovery as compared with baseline, and, compared with controls, have significantly less REM time, REM%, and change in REM time and REM% on early post-deprivation nights.

The two patient groups also differed in their pattern of stages III and IV during recovery. Considerable overlap existed in REM compensation between actively ill schizophrenics and controls. Additional information suggests that REM compensation may be related to Rod and Frame testing: the more field independent a subject is, the better REM compensator he is.

2. 5-Hydroxytryptophan and Schizophrenia

5-Hydroxytryptophan was studied at doses of 200-60000 mg/24 hr with MK486 in 18 chronic schizophrenic patients. EEG sleep studies revealed:

(a) REM sleep was reduced at all doses; no rebound was found with 5HTP withdrawal; (b) REM latency was increased at all doses and (c) NREM sleep was increased at low doses, decreased at high doses, and decreased still further during withdrawal.

J. Sleep in Gilles de la Tourette's Syndrome

The sleep of 6 Tourette patients (drug-free and while taking haloperidol) was compared with 9 normal volunteers. The untreated patients had 30% less delta sleep, which returned to control values when they received haloperidol.

K. A Case of Sleep-walking and Somnambulistic Homocide Associated with Psychotropic Drugs

The patient is a 44 year-old white female who had a history of one sleep-walking episode in adolescence but none after that until age 40. She also had had enuresis until age 16. Except for brief psychiatric treatment at age 16 for enuresis and anxiety, she had no psychiatric history until age 39, when she developed a florid psychosis with persecutory symptoms. She was diagnosed as schizophrenic, hospitalized for 2 weeks, and treated with phenothiazines. A year later she experienced a return of symptoms and resumed treatment on an outpatient basis. Because of insomnia, she received thioridazine and Triclos (a chloral derivative) at bedtime. At 3 a.m. on the third night of this regimen, she arose from her bed and stabbed her daughter to death, possibly while asleep or in a confused state. She was found to be hallucinating and delusional when examined the next morning and diagnosed as suffering from paranoid schizophrenia. At her trial she was found innocent on the grounds of insanity and hospitalized under court order.

On phenothiazines and psychotherapy, her psychosis slowly cleared. However, she was noted to sleep-walk 2-4 times per week. Physical investigations, which eventually included EEG's with nasal-pharyngeal leads, A CAT scan, and a pneumoencephalogram, were all normal. Dilantin and tricyclic antidepressants had no effect on her sleep-walking.

After 4 years in the hospital, she was transferred to our unit, and thioridazine 300 mg q.h.s. was discontinued. During a one-month drug-free period, 11 all-night sleep EEG's along with continuous video monitoring were carried out. There were no sleep-walking episodes. However, when treated with thioridazine 300 mg q.h.s. and chloral hydrate 1000 mg q.h.s. she sleep-walked on 5 of the 6 nights recorded. On thioridazine 300 mg q.h.s. alone, she walked one night in four; on chloral hydrate 2000 mg alone, she did not walk during three nights of recording. All sleep-walking episodes occurred out of slow-wave sleep.

These results suggest that she sleep-walked only on psychotropic medications, particularly thioridazine. This case is similar to those reported by Huapaya (Am. J. Psychiatry 133: 1207, 1976) of somnambulism secondary to bedtime doses of methaqualone in two cases, chlorprothixene in another, and the combination of methaqualone and thioridazine in the fourth. In all

cases sleep-walking could be abolished by either reducing or eliminating the bedtime dose. Two of the cases had childhood histories of sleep-walking, as did our own. As well, Flemenbaum (Am. J. Psychiatry 133:570-572, 1976) has reported cases of pavor nocturnus, another disorder associated with stage 4 sleep in patients rreceiving large bedtime doses of tricyclics or antipsychotics. The mechanism of this phenomenon, which may not be uncommon, is unknown. We are presently analyzing our patients' sleep EEG's.

Because of continued interest in issues of efficacy and safety of long-term hypnotic administration, we have performed a study of EEG-defined sleep, subjective reports of sleep, and daytime functioning in 10 insomniacs. These subjects were chosen on the basis of a report of chronic sleep disturbance and the absence of medical, psychiatric or diagnosed sleep-related pathology.

The major EEG effects of flurazepam (30 mg HS for 28 nights) were an increase in total sleep time during the period of days 13, 15, and 27, 28 ($p<0.05$) but no significant decrease in sleep latency or intermittent waking time. REM percentage was significantly decreased ($p<0.05$), particularly on days 13 and 15, as was percentage stage 4 ($p<0.05$) on days 27 and 28. Subjective reports of sleep indicated improvements in quality of sleep, difficulty getting to sleep, and sleep latency, primarily in the first week of treatment. There was a non-significant increase in perception of total sleep time. Blood concentrations of desalkylflurazepam rose from the general range of 18.1-62.4 ng/ml on the morning after one dose to 41.2-169.4 on day 13 to 41.0-179.9 on day 26. In the daytime there was no significant alteration in sleepiness, activation, dysphoria or depression, although there were significant decreases in the euphoria scale ("sense of well-being") during chronic drug administration and withdrawal. Daytime testing showed marked impairment of time to do letter cancellation test and a variety of cognitive tasks, including serial learning, prompted recall and free recall tasks. A questionnaire of physical symptoms showed no major changes in the 10 subjects. A single subject, who had mild respiratory changes before the study, developed a sleep apnea syndrome by the second night of flurazepam, which cleared upon cessation of the drug.[1]

L. Flurazepam-induced Sleep Apnea Syndrome in an Insomniac

Sleep, EEG, and respiratory measures were examined in a 38-year-old man with a long-standing history of insomnia and daytime sleepiness. He was found to have seven to 18 primarily obstructive apneas per night on four baseline recordings, a finding not generally considered to be indicative of pathology. On the first two nights on which he received 30 mg of the benzodiazepine hypnotic flurazepam, there were 22 and 100 apneas, and during the daytime he became extremely sleepy. Upon cessation of medication, his clinical condition improved, and the number of apneas decreased to 11 and 6 on withdrawal nights 4 and 6. Although respiratory depression is neither invariable nor unique to flurazepam, this case suggests that it may be a clinically significant problem with recommended oral doses in some individuals.

IV. Major Findings: Normal Studies

A. Effects of Sleep Stage and Stimulus Intensity on Auditory Evoked Responses

The purpose of this study was to evaluate the effect of sleep stage and stimulus intensity on the auditory evoked response (AER). Nine normal volunteers (age 18-22; 6M, 3F) slept in the sleep lab for three nights each; the first night was adaptation and was not included in any calculations. Four intensities of clicks (50, 60, 70 and 80 dB) were generated by the LINC computer in pseudo random order. EEG was sampled at 250 Hz for 1 second and AER was computed on line for each intensity for each minute of the night. Each minute was visually scored for sleep stage. AER from the minutess of each sleep stage were then averaged. AER amplitude tended to increase little from 50 to 80 dB in waking subjects but increased markedly in sleeping subjects in stage 3 and 4 ($p<0.05$, 2-way analysis of variance). Rapid eye movement (REM) and stage I sleep had small amplitude AER's in comparison with other sleep stages.

Individuals who showed decreases in amplitude at high intensities while awake slept significantly longer during the experimentsl night ($r=.76$, $p<0.05$). The loss of inhibitory mechanisms during stages 3 and 4 is well known. It is therefore not surprising to see the disappearance of the paradoxical AER amplitude reduction at high intensities and a more linear EP amplitude/stimulus intensity function; stage 1, 2 and REM sleep resembled the waking state. Of interest was the finding that waking AER amplitude/ stimulus intensity functions predicted sleep duration in this noisy environment. This suggests that individual differences in sleep duration may be related to differences in inhbition of sensory processing.

B. EEG Cortical Map in Sleep

Computer-generated cortical maps of power spectral estimates derived from 16 leads were drawn based on daytime sleep recordings in four normal epochs from awake, stages 1-4 and REM sleep in each volunteer. EEG leads were placed on the left hemisphere and midline according to the 10-20 system with four additional interpolated posterior locations. Spectral estimates with 1 Hz resolution and several frequency bands (delta 2-4; alpha 8-12, beta 13- 18) were analyzed with a two-way ANOVA (lead by sleep stage). Delta activity was relatively uniform and of low amplitude in awake, eyes-closed subjects and REM. Delta power increases at the vertex in stage 1 which, with successive nonREM sleep stages, both progresses in power and enlarges radially to the intraparietal sulcus posteriorly and the superior frontal gyrus anteriorly. Beta activity in awake subjects was low and maximal parietally. Stages 1 and REM showed even lower and more uniform distribution. Stage 2 showed the greatest power, concentrated at the vertex, with stages 3 and 4 diminishing. Alpha activity was expectedly maximal occipitally in awake subjects, but surprisingly a frontal area appears in slow-wave sleep. All findings were confirmed by a two-way interaction. These data indicate that the sleep stages are not electrophysiologically uniform across the cortex and open the possibility for a new approach to the diagnosis of sleep and psychiatric disorders.

V. Major Findings: Animal Behavior

A. Deprivation of Rapid Eye Movement Sleep and Nesting Behavior in Rats

The effects of REM deprivation for four days on nesting and locomotor activities were studied in male rats. Four groups of animals were tested. (a) Experimental animals (SP), deprived of REM on a small (6.5 cm) pot; (b) large pot (LP) control, maintained on large (12.5 cm) pots; (c) stress group, forced swimming for 1 hr/day; (d) normal controls. The results indicate, as predicted, increased utilization of nesting material by the experimental (SP) group in the post-REM deprivation period as compared with normal controls (464 cm for SP, 94 for normals, $p<.05$). Nevertheless, the SP group did not differ significantly from LP (356 cm) or stress (133 cm). Since LP does suffer some REM deprivation, though less than SP, the results suggest that increasing levels of REM deprivation may increase a drive-related behavior in rats.

B. EEG Documentation of a Method for Sleep Depriving Rats

There is a need for a relatively simple method of sleep deprivation which is supported by EEG data and which can process a number of animals at once with a minimum of labor. We shall describe such a technique, which consists of keeping rats awake by maintaining them in slowly rotating cyclinders.

Rats were placed in two Nalgene cylinders (68.6 x 35.6 cm), each of which is divided into six compartments. An electric motor causes them to rotate at 3 RPM. A strip of tubing on the floor of each compartment provides an obstruction over which rats must walk, so that they cannot slide along.

In order to document the state of consciousness of rats undergoing this procedure, we recorded the EEG and EMG of four rats in the moving cylinders for 24 hours and compared them to seven enimals in a stationary cage. The moving cylinder reduced sleeping time from 47.0% of the 24-hour period to only 3.8% ($p<0.001$). The small amount of sleep which did occur was made up of both REM and NREM sleep. The percentages of total sleep time did not differ between the two conditions. Thus, there was no selective effect on one particular sleep stage, and instead total sleep time as a whole was markedly reduced.

C. A Signal Analysis of the Rat Sleep EEG: The Application of Continuous Frequency Analysis

Automated rat sleep analysis focuses on the statiscally regular waveforms of the EEG, such as theta and delta rhythms. Such stochastic processes can be quantified in several manners. Time domain statistics such as auto- and cross-correlations produce outputs that are difficult to use and are best performed in software. Frequency domain statistics like spectral density accurately quantify the sleep state by power

frequency distributions but also require sophisticated computer processing. Continuous frequency analysis, using passband filtering, accurately measures signal power in an on-line fashion and employs relatively inexpensive hardware to estimate power by integrating the sqaure of the signal. This method differs substantively from other previously reported systems which rely on signal amplitude analysis. Comparison of this system with a human scorer indicates high degrees of validity and reproducibility.

D. Sleep in Rats with Bilateral Habenular Lesions

The diencephalic habenula nuclei (HBN), which receive afferents from several limbic structures, are thought to send an inhibitory pathway to the dorsal raphe nuclei (DRN). Thus electrical stimulation of the HBN has been reported to decrease DRN cell firing, and bilateral lesions of the HBN may lead to a 60% increase in 5 HIAA content of the DRN (manuscript in preparation). These lesions have been associated with increased daytime motor activity in rats (manuscript in preparation). Because of longstanding interest in the role of the raphe in sleep regulation, we have examined the effect of bilateral HBN lesions on sleep in the rat.

Ten male 200 gm Sprague Dawley rats received bilateral radio frequency lesions of the HBN, and 11 had sham operations. They were then implanted with EEG and EMG electrodes, and 8 hour recordings starting at 8:30 a.m. were performed one week later. Analysis of the data revealed no significant changes in sleep latency, total sleep, or any sleep stage measure.

A lesion producing relatively drastic changes in dorsal raphe cell activity might well be expected to influence sleep, and it is thus surprising that no such change was found. Alternative explanations for this might be that (1) lesion effects are seen only at other parts of the circadian cycle; or (2) during the week between lesioning and recording, the nervous system develops tolerance to perturbations from altered serotonin metabolism. One argument in favor of the latter hypothesis comes from reports of tolerance to the effects on sleep of the serotonin synthesis inhibitor parachlorophenylalanine.

VI. Studies of Human Memory:

A. Effects of Arecoline or Scopolamine Prior to learning.

We determined the effects of arecoline and scopolamine on categorized serial learning in normal volunteers, all of whom were college students or recent college graduates.

The results indicated that arecoline (4 mg) significantly reduced the number of trials to criterion as compared with saline (mean 3.76 vs. 5.18 trials, $p<.01$). In contrast, the mean number of trials following scopolamine (0.5 mg) was 6.35 ($p<.01$, compared with 5.18 on methscopolamine-saline control). Administration of arecoline 6 mg, however,

reversed the effects of scopolamine 0.5 mg (mean number of trials was 4.92, which was significant, $p<.01$, compared with scopolamine alone).

We also found that the major effects of arecoline and scopolamine were seen in naturally "poor" performers rather than "good" performers. The correlation between baseline, placebo performance and change on arecoline was $r=0.93$, $p<.001$, indicating that greater improvement was seen in those subjects who took the most trials to criterion on baseline; for scopolamine, the correlation was $r=0.5$, $p<.05$, indicating that the greatest decrement in performance was seen in those who took the most trials to criterion.

B. Effects of Arecoline Following Learning

We showed that administration of arecoline immediately after learning facilitates later recall. Eight subjects (5 males, 3 females, mean age 24) were tested on two nonconsecutive days. Prior to the experiment, an intravenous needle was inserted in a forearm vein and attached to a ten-foot catheter extending out of the room so that drugs could be administered without the awareness of the subject. Following administration of methscopolamine (0.3 mg iv), subjects heard a list of words, nine groups of five words each. Three of the nine groups consisted of highly related (associated) words, three groups of weakly associated words, and three groups of randomly associated words. The groups were presented in order: first a highly related group, then a weakly associated group, then a randomly associated group, until all nine groups had been presented. After listening to each group of five words, subjects were asked to think of and say a word which defined or organized that word cluster. Immediately at the end of the session, subjects received a twenty minute infusion of either arecoline (2 mg in 20 ml of saline) or placebo (20 ml saline). Recall testing began 1 hour following the onset of the infusion. Subjects were tested first under free recall conditions and, then, under "cued" recall conditions (after having been presented with their own, self-generated cue word).

Compared with placebo infusion, arecoline significantly improved recall of the presented word; all three types of word sets--highly associated, weakly associated, and randomly associated--were better recalled ($p<.05$, $F=7.8$, $df=1,7$). This was true under both free and cued recall conditions. Interestingly, the arecoline effect appeared to be mediated by an increase in the number of clusters recalled ($p<.01$, $F=13.6$, $df=1,7$) rather than an increase in the number of words per cluster. While cued recall was better than free recall ($p<.001$, $t=29.6$, $df=1,7$), and highly associated word sets were better recalled than weakly or randomly associated words, the facilitory effect of arecoline did not interact with these factors.

C. Effects of Choline Chloride on Human Learning

To study the effects of choline chloride in man, we administered an elixir containing 10 gm or placebo on separate days. Ninety minutes later, we began the session with two different tests: uncategorized serial learning and a selective reminding task.

Choline administration improved performance on both tests. On the uncategorized serial learning tasks, subjects learned to recite ten unrelated words in correct order; following each trial, the entire list was repeated by the experimenter. The number of trials to criterion was 5.7±0.7 after choline and 6.1±0.9 after placebo ($p<.05$, two-tailed t-test). On the selective reminding task, subjects learned a 12 word list of words, half of which were high image words (such as table) and half of which were low image words (such as virtue). Following each trial, the investigator reminded the subject of the words missed on the last trial, but did not repeat the words correctly remembered. As might be expected, high imagery words were learned in fewer trials on placebo than low imagery words, and choline did not alter the number of trials required for high imagery words. Choline administration, however, significantly reduced the number of trials required to learn low imagery words from 9.1±0.9 to 6.8±0.5 ($p<.01$).

The effects of choline were significantly correlated with performance on placebo, indicating a greater effect in "poor" performers than "good" performers. The change induced by choline was significantly correlated with performance on placebo on the uncategorized serial learning task ($r=0.59$, $p<.05$, $N=10$).

D. Effects of Lecithin on Memory and Plasma Choline Levels in Normal Volunteers.

A pure grade of lecithin (phospholipon 100 - about 95% phosphatidylcholine) was administered to 15 normal volunteers in doses of 20, 40, and 60 gm. A dose-dependent elevation of plasma choline was noted, which persisted for at least 23 hours. The highest dose produced some nausea and GI symptoms. No overall change in memory and learning was noted. The "poor performers," however, again showed a significant dose-dependent increase in performance at 20 and 40 gm as compared with placebo.

E. Cholinergic Therapies in Alzheimer's Disease

Preliminary data fails to show a significant effect of lecithin in patients with clinically defined Alzheimer's disease. Nevertheless, it does appear that the combination of lecithin and THA (an oral anticholinesterase) may improve cognitive performance. Further studies are underway.

VIII. Thermoregulation in Patients with Primary Affective Illness

The regulation of body temperature may be another CNS function which may be under the partial control of acetylcholine. We studied the hypothermic response of arecoline in a group of 17 normals and 10 primary affective patients in complete remission who were drug free for more than 2 weeks. Arecoline was administered as a slow IV drip (.066 mg per min, total dose - 2 mg every 10 min). There was no difference between normals and patients with respect to the hypothermic effect of arecoline.

Our data suggests that patients with primary affective illness appear to be hyper-responsive to pharmacological challenge with cholinergic agonists in two out of three measures tested; i.e., REM sleep induction, and pupillary miosis. They did not differ from normals with respect to the hypothermic effect of arecoline. The regulation of temperature, like blood pressure, is, of course, a very complex central and peripheral nervous system function. Thus, it may not be an appropriate physiological system to measure specific cholinergic effects.

Our aim has been to develop pharmacological challenge strategies utilizing physiological endpoints that could be objectively measured. Our data strongly supports the existence of a hyper-responsive cholinergic system in affective illness, both during the depressed and euthymic (remitted) states.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00098-07 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Children and Grandchildren of Patients with Depressive Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.4	1.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to determine the frequency of affective disorders in the children of adults with unipolar and bipolar depressive illness, and to identify biological markers of a genetically-transmitted predisposition to affective disorders, and to study the interaction between genetic predisposition and environmental factors in children with affective disorders. Our present project is a refined replication of our pilot project reported last year. The major changes involve the inclusion of two control groups as well as a blind interviewer and rater.

Project Description:

Objectives: 1) to determine the frequency of affective disorders in the children of adults with unipolar and bipolar depressive illness; 2) to identify biological markers of a genetically-transmitted predisposition to affective disorders; and 3) to study the interaction between genetic predisposition and environmental factors in children with affective disorders.

Methods Employed: We are studying three groups of children: 1) 20 children of parents hospitalized at NIMH with a primary affective illness; 2) 10 children of patients hospitalized at NIH because of a physical illness and screened for any affective disorder; and 3) 20 children of "normal" parents with no known physical or mental disorder. All children are seen twice, four months apart. 4) There was also a comparison group of 11 children of parents with chronic physical illness, not screened for mental illness.

A social worker obtained at each visit a history from the parents and administered the Achenback Scale for childhood psychopathology.

At each visit the child had a psychiatric interview. The interview was conducted by a blind interviewer and rater, while another investigator observed through a one-way mirror. The interviews were structured, and followed a standard inventory of topics aimed at eliciting various types of psychopathology such as affective disorders, anxiety reaction in childhood psychosis, delinquency and minimal brain dysfunction. A portion of the interview is aimed at eliciting fantasy material by the use of free drawings, story telling, and free reporting of dreams, T.V. stories, and movies. Other methods used in child psychiatry such as the three wishes and the Despert Fables were also employed. Following the interview, the children were rated on the CPRS scale of the ECDEU as well as on a global rating scale for affective disorders constructed for this purpose and used by the investigators for many years. All of the above mentioned procedures pose no hazards for the children. In addition, after each visit the children were rated on the Weinberg criteria for depression in children.

Major Findings: The children in the manic-depressive group had significantly more depression on all interviews than the two control groups, according to the Weinberg Criteria. The children in the two control groups did not differ from each other in the incidence of depression. However, both control groups had significantly less depression than the children of manic-depressive parents.

As for the comparison group, of the 10 parents with chronic physical illness 7 had also a concommitant major mental illness, mostly affective and in the "depressive spectrum." The 11 children in this group did not differ significantly from the children of the manic-depressive parents in the incidence of depression.

Significance to Biomedical Research and the Program of the Institute: This study confirms the findings in our pilot study about the high incidence of significant depressive illness in the children of parents with affective disorders. The question still remains, however, whether the depression in the offspring is genetically determined or mainly due to environmental factors. The answer to this question may have to await the finding of a biological marker for affective disorders.

Proposed Course of Project: The 20 children of manic-depressive parents and 20 matched control children are currently being reevaluated using the above mentioned criteria and the revised DSM-III diagnostic system.

Publications:

Cytryn, L., McKnew, D.H., Jr., Bartko, J.J., Lamour, M., Hamovit, J. and Bunney, W.E., Jr.: Offspring of patients with affective disorders II. Journal of the American Academy of Child Psychiatry. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00100-06 BP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Biobehavioral Aspects in Childhood and Adolescent Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.20	.95	4.25

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An inpatient program with selected overnight stays, for childhood and adolescent neuropsychiatric disorders is ongoing. The condition currently under study is that of hyperactive children (HAC). Pharmacological compounds under study in these disorders include methylphenidate, amphetamine, piribedil, L-dopa, tryptophan, Mianserin, and clorgyline. Pharmacokinetic studies with clinical responses are ongoing. Central neurotransmitters and their metabolities are being studied in plasma and urine. Amphetamine half-life in children is about one-third that of adults. Behavior and motor activity responses to d-Amphetamine occur during the absorption phase as determined by serial plasma amphetamine following a single dose. Urinary 3-methoxy-4-hydroxy-phenyl glycol shows a time-related decrease during treatment with d-amphetamine; homovanillic acid is unchanged. In general, norepinephrine metabolites in urine are decreased after two weeks of d-Amphetamine vs. placebo and dopamine metabolites are not. Tyramine and its metabolites are also decreased following d-Amphetamine, whereas phenylethylamine is greatly increased following d-Amphetamine. Piribedil is safe but clinically ineffective in HAC. L-dopa is minimally clinically effective in HAC.

Project Description:

Objectives: The purposes of this program are broad. An objective is to gain new knowledge of the central nervous system (CNS) of children and adolescents with especial reference to maturational changes and neuropsychiatric disorders. Compared to the neurobiology known in adult neuropsychiatry, much less is known regarding the neuropsychiatric disorders of children.

Several principles are important to keep in mind in the study of children versus adults: 1) morphological brain maturation is age related, i.e., the reticular activating system, of considerable importance in psychobiology, is not fully myelinated until ages 10 to 20; 2) children may have more rapid and clear pharmacological responses, both therapeutic and toxic; 3) no clear history of pre-adolescent "addiction" to compounds, i.e., amphetamine, which can effect unequivocal addiction in adolescents; 4) most behavioral symptoms in children that respond to pharmacological treatment have more of an apparent organic contribution than those of adults, i.e., motor hyperactivity, enuresis, poor attention span in children versus complex emotional and cognitive aberrations in adults; 4) clinical symptomatology is more variable and fluctuating in children than usually seen in adults, i.e., rating scales for endogenous depression in adults are more easily constructed to measure a "constellation" than is the case for childhood depression; well formed delusions and hallucinations are not so common in young psychotic children as in adults.

A number of studies, pharmacological and otherwise, have been undertaken to elucidate cerebral metabolism in specific conditions.

Methods Employed: An inpatient and day patient program for children and adolescents, involving selected overnight stays, is ongoing on the 3 East Nursing Unit. Children who are hyperactive, aggressive and impulsive, and who have learning difficulties have been admitted in order to study a carefully defined population of HAC. Children and adolescents with other conditions have also been studied.

All children are thoroughly evaluated by medical, psychiatric, and psychometric examinations with all routine and other indicated procedures and clinical laboratory studies. Children also receive a psycholinguistic examination in collaboration with NINCDS. Neurological examinations are scored carefully according to a rating scale (PANESS). Several clinical and behavioral rating instruments have been utilized.

Pharmacological compounds, both standard and those previously unused in children, are being studied. Serial plasma pharmacokinetic data are being generated for d-Amphetamine (d-AMPH). These data are studied in conjunction with motor activity, behavior, cognition, speech, temperature, and cardiovascular response. Piribedil, a specific dopamine (DA) agonist, and L-dopa have been given to HAC. Mianserin, a norepinephrine (NE) agonist, tryptophan (TP), a precursor of serotonin (5HT), and clorgyline, a monoamine oxidase (MAO)A inhibitor, have been administered to HAC as clinical trials.

Motor activity is measured by an ambulatory activity monitor with solid state memory which measures individual motor movements via a pendulum acceleration system per unit of time and set at a desired sensitivity for the particular study. At any time the instrument can be read into a computer for a print-out. Behavioral changes in HAC are measured via Conners' Teachers' Rating Scale (CTRS). Cognition is measured by a continuous performance task (CPT) in which errors of omission and commission can be scored in terms of differing time intervals. Time intervals can also be increased or decreased in relationship to accuracy of response.

D-AMPH is measured by a radioimmunoassay (RIA) and gas chromatograph mass spectrometry (GC-MS). Biochemical studies include 24-hour urine collection to study NE, 3-methoxy-4-hydroxyphenylglycol (MHPG), vanillylmandelic acid (VMA) normetanephrine (NMN), DA, homovanillic acid (HVA),3, 4-dihydroxy-phenylacetic acid (DOPAC), 3-methoxytyramine (3-MT); tyramine (TY), parahydroxyphenylacetic acid (PHPA); and phenylethylamine (PEA) and phenylacetic acid (PAA). Plasma pharmacokinetics of pharmacological compounds are being ascertained. Plasma NE, MHPG, NMN and VMA changes as they relate to plasma d-AMPH levels have also been studied. Neurophysiological studies include routine and sleep EEG's and EMI scans when indicated. Average Evoked Response (AER) studies are being conducted as they relate to HAC in drug-free and treated conditions. Psycholinguistic changes are also studied in relation to d-AMPH, piribedil, and TP effects. Paired associate learning has also been assessed in different drug conditions. Chronic effects of d-AMPH (2 weeks) are being studied with regard to pharmacokinetics and clinical response, particularly in terms of an evidence for tolerance or supersensitivity and effect on catecholamine metabolism (as manifested by changes in plasma NE and dopamine-beta-hydroxylase (DBH) and platelet 5HT and MAO.

Major Findings: Significant correlations exist between community teachers' and NIH teachers' CTRS ratings of behavior.

Serial plasma pharmacokinetic data indicate that children reach a peak plasma level of d-AMPH within 3-4 hours (h) of an initial dose; however, as much as 70-80% will remain in the serum at 5-6 h when behavioral effects have largely dissipated. Mean apparent elimination half-life is 6.8 $\pm$ 0.5 h. Test-retest studies of individuals indicate that both pharmacokinetic data and clinical response data is highly replicable. Clinical responses have also been studied after use of sustained release capsules. This preparation produces a slower rate of absorption and a more plateau-like, longer lasting peak level, but does not give a prolonged clinical response. Socially appropriate behavioral change and motor activity change is maximal at 1-3 h after administration of a single dose (0.5 mg/kg) of d-AMPH which corresponds to its absorption phase. This data indicates that the clinical changes may be related to removal release of catecholamines and the subsequent depletion of their stores to replacement by a "false" neurotransmitter metabolite of AMPH, or to alteration in receptor sensitivity. Higher single doses (1.0 mg/kg) effect similar clinical responses, but less so. Piribedil is safe, but clinically ineffective for HAC. Preliminary results indicate that neither TP nor valine (a mental amino acid which competes with TP and inhibits its crossing the blood-brain barrier) result in behavioral response after a single dose.

Studies of urinary metabolites indicate that day and night excretion of MHPG and HVA are not significantly different; however, d-AMPH after eight days is associated with significantly lower MHPG levels and the data indicates that behavioral response may be associated with the decrement in MHPG. Urinary HVA is unchanged. These biochemical findings have been replicated in a subsequent group. PEA excretion is markedly elevated following two weeks of treatment with d-AMPH.

HAC are not significantly different from normal controls with regard to plasma NE and DBH but do have significantly more neurological soft signs by PANESS examination. Plasma NE is significantly correlated with anxiety as well as being significantly elevated at 2-3 h following a single oral dose of d-AMPH. The change in plasma NE correlates with the change in plasma d-AMPH. The elevated plasma NE is associated with increases in blood pressure and pulse and is dose-related.

Studies evaluating CPT are now underway; d-AMPH is effective and piribedil and L-dopa are minimally so. AER studies are preliminary. HAC with higher levels of soft signs have more abnormal EEG's, more minor physical anomalies, lower full scale IQ (WISC-R) and a greater number of errors on the Bender. Data from psycholinguistic evaluations are being prepared for publication.

Significance to Mental Health Research: Though childhood neuropsychiatric disorders, and particularly HAC, have been considerably studied in the last few years, there are many diagnostic, psychopharmacological, and psychobiological questions to be answered. Many studies in the past in child psychiatry have been related to psychological, psychodynamic issues. As regards HAC, obsessive-compulsive children, enuretics, Gilles de la Tourette's syndrome, Anorexia Nervosa, psychoses, and autism, an increased interest in psychopharmacology has emerged. Though methylphenidate and AMPH give positive responses in 80% of well diagnosed HAC, the pharmacokinetics and metabolism of these drugs had not been closely studied in children. The relationship between such basic pharmacological knowledge and clinical effects have been understudied in children in general. More importantly, for the future, basic biological factors in childhood neuropsychiatry which might elucidate the psychopharmacological responses are, at this point, only hypotheses. The degree to which these hypotheses are validated or refuted could play a significant role in understanding the biological contributions to childhood neuropsychiatry.

Proposed Course of Project: We plan to conduct this program over an indefinite period.

Publications:

Caine, E.C., Margolin, D., Brown, G.L., and Ebert, M.H.: Gilles de la Tourette's syndrome, tardive dyskinesia, and psychosis in an adolescent. Am. J. Psychiatry 135: 241-243, 1978.

Brown, G.L., Ebert M.H., and Hunt, R.D.: Plasma d-amphetamine absorption and elimination in hyperactive children. Psychopharmacol. Bull. 14(3): 33-35, 1978.

Rapoport, J.L., Mikkelsen, E.J., Ebert, M.H., Brown, G.L., Weise, V.K., Kopin, I.J.: Urinary catecholamines and amphetamine excretion in hyperactive and normal boys. J. Nerv. Ment. Dis. 166(10): 731-737, 1978.

Brown, G.L., Hunt, R.D., Ebert, M.H., Bunney, W.E.Jr., Kopin, I.J.: Plasma levels of d-amphetamine in hyperactive children: serial behavior and motor responses. Psychopharmacology 62: 133-140, 1979.

Brown, G.L., Ebert, M.H., Mikkelsen, E.J., Hunt, R.D.: Clinical pharmacology of d-amphetamine in hyperactive children. In: Gottschalk, L.A. (ed.), Pharmacokinetics of Psychoactive Drugs--further studies, New York, Spectrum Publications, pp. 137-153, 1979.

Brown, G.L., Ebert, M.H., Mikkelsen, E.J., Hunt, R.D.: Behavior and motor activity response in hyperactive children and plasma amphetamine levels following a sustained release preparation. J. Am. Acad. Child Psychiatry 19: 225-239, 1980.

Langer, D.H., Rapoport, J.L., Brown, G.L., Ebert, M.H., Bunney, W.E. Jr.: Questioning a dopaminergic hypothesis. Am. J. Psychiatry 138(4): 537, 1981.

Mikkelsen, E.J., Lake, C.R., Brown, G.L., Ziegler, M.G., Ebert M.H.: The hyperactive child syndrome: Peripheral sympathetic nervous system function and the effect of d-amphetamine. Psychiatry Res. 4(2): 157-169, 1981.

Brown, G.L., Ebert, M.H., Hunt, R.D., Rapoport, J.L.: Urinary 3-methoxy-4-hydroxyphenylglycol (MHPG) and homovanillic acid (HVA) response to d-amphetamine in hyperactive boys. Biol. Psychiatry, in press.

Langer, D.H., Fletcher, J.C., Brown, G.L., Nee, L.E., Smith, M.A.: Ethical considerations in psychological research in children. In: Greenhill, L., Shopsin, B. (eds.). The Psychobiology of Childhood: Profiles of Current Issues, New York, Spectrum Publications, in press.

Brown, G.L., Ebert, M.H.: Catecholamine metabolism and hyperactive children. In: Lake, C.R., Ziegler, M.G. (eds.). Norepinephrine: Clinical Aspects, Baltimore, Williams and Wilkins, in press.

Mikkelsen, E.J., Brown, G.L., Millican F.K., Rapoport, J.L.: Neurologic status in hyperactive, enuretic, encopretic, and normal children. J. Am. Child Psychiatry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00101-05 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Childhood Enuresis: Clinical Response to Placebo, Imipramine and Methscopolamine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	J. Christian Gillin, M.D.	Research Psychiatrist	BP	NIMH
	Richard J. Wyatt, M.D.	Director	SMR	NIMH
	William E. Bunney Jr., M.D.	Chief	BP	NIMH

COOPERATING UNITS (if any)
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LAB/BRANCH
Biological Psychiatry Branch

SECTION
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INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
00	00	00

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project has been discontinued

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00102-04 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Lithium Response in Children of Lithium Responding Manic Depressive Parents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP NIMH
	Elliot S. Gershon, M.D.	Chief, Section on Psychogenetics	BP NIMH
	Joel R. Hamovit, M.S.W.	Research Social Worker	BP NIMH
	Walter Sceery, J.D.	Social Worker	BP NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH
Biological Psychiatry Branch

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Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.0	0.6

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☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of lithium in emotionally disturbed children having at least one manic-depressive lithium responsive parent will be examined. Thirty children, ages 6-12, of both sexes, having psychiatric symptoms that are in some way incapacitating and are of major concern to the child, parent, or teacher will be selected. Children will be given lithium and placebo, in a double-blind, cross-over design for 16-18 weeks. Behavior ratings will include: parent's and teacher's assessment, psychophysiological measures, child interview, and cognitive testing. The lithium administration will be monitored by an "unblind" co-investigator.

This project has been discontinued.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1) To test the hypothesis that some of the offspring of lithium responding parents will prove clinically responsive to lithium; 2) to test the hypothesis that children with an affective and/or cyclic component to their psychopathology will be better responders; 3) to see if the response to lithium may possibly be a genetic marker for manic-depressive illness; and 4) to identify the children at risk with early prodromal disorders who respond to lithium, prior to the outbreak of manic-depressive illness.

Methods Employed: The 30 patients will be admitted to the study upon recruitment from the NIMH Outpatient Department (OPD) as well as from the local psychiatric community. The fact that the parents are indeed lithium-responding manic-depressives will be independently verified on the basis of SADS interviews and RDC diagnoses.

The patients will be selected on the basis of having sufficient psychopathology for it to be incapacitating and to be of concern to their parents and/or the school authorities. Any children with significant brain damage and/or an IQ below 80 will be excluded from the study. The patients will be ages 6-12 of both sexes, and with no regard for their socio-economic status. These patients will be admitted to the Outpatient Clinic for the duration of the study. Patients of special interest may be retained in the OPD for follow-up treatment and study. Other patients will be returned to their previous source of care or referred to local psychiatrists, as needed.

Their work-up records will be handled as described in the Handbook for Staff Physicians.

Admission Work-Up:

Parents: A structured history will be obtained. In addition, the parents will be requested to fill out the following questionnaires: 1) Deviant Behavior Inventory (DBI); 2) the Achenbach Scale.

Patients: A diagnostic evaluation will be obtained including a structured interview and a routine physical exam. On the basis of all available information, a clinical diagnosis based on DSM-III will be made. The following rating scales will be filled out: 1) Chidren's Psychiatric Rating Scale (CPRS of the ECDEU); and 2) Children's Affective Rating Scale (CARS).

Blood will be drawn for creatinine, BUN and T_3, T_4 and TSH to rule out renal or thyroid pathology. These tests will be repeated at the end of the study.

The physiological test, Average Evoked Response (AER), will be administered.

Teachers will fill out the brief Conners' Teacher Rating Scale.

The thirty patients will be randomly assigned to two groups (I and II). Group I will be treated as follows: Approximately four weeks placebo, four weeks lithium, four weeks placebo, four weeks lithium. The procedure will be reversed in Group II.

Nature of Procedure: Lithium will be administered in blind fashion except that a case "coordinator" will be unblind. The first lithium administration will begin with the mean child dose for that age and will be adjusted until a blood level between .8 and 1.2 mg/liter is obtained. The case coordinator will adjust the number of tablets for a matched placebo case in parallel with adjustment for active drug.

Side effects will be monitored. The second period of lithium administration will begin with that child's effective dose.

1) The patients will be seen on a weekly basis for evaluation and administration of medication. At each visit the following procedures will be carried out: A brief structured interview of 15-30 minutes with a special focus on the target symptoms. The interview will be rated on the target items of the CPRs and the CARS. Lithium levels will be obtained in serum. A global rating scale will be filled out by the interviewer on each diagnosis made. 2) The parents will rate their target symptoms weekly and a global rating will be done by the parent interviewer also.

The AER will be repeated on a schedule regulated by the unblind lithium administration.

Major Findings: To date, a total of six patients have completed the study and five more are being evaluated. Table II gives the children's diagnoses and the children's response to lithium, as rated independently by the child's interviewer, the parent's interviewer and the teacher.

Two girls, (A & B) ages 8 and 12, diagnosed as having a bipolar affective disorder, mixed, responded to lithium on both the child as well as parental ratings. B had a dramatic response to lithium in both manic and depressive symptoms, but the mother kept occasionally giving lithium during placebo periods (verified by positive lithium blood levels), making statistical evaluation impossible. A boy (C) age 11, diagnosed as having an attention deficit disorder with hyperactivity and overanxious disorder was rated as lithium responsive on the basis of the parents' reports, but not on the reports of the child's interviewer. The teacher's rating closely approached significance. An 11-year-old boy (d) with three diagnoses: Major depressive disorder, recurrent, attention deficit disorder with hyperactivity and overanxious disorder was rated as lithium responsive by the child's rater only on overanxious disorder, but not on the other diagnoses. The parent's and teacher's ratings were negative for all diagnoses. The two remaining children showed no response to lithium on any rating: (E) a boy age 12 with an attention deficit disorder with hyperactivity and cyclothymic disorder, (F) a boy aged 10 with encopresis and major depressive disorder, recurrent.

EP Results: The full set of EPs was obtained on five children; one child (B) received only the baseline and two lithium sessions. For the purposes of predicting lithium response, we used the average of the P100 amplitude/intensity slope for the two placebo sessions in five children and the baseline in the sixth (b).

The evaluation of the efficacy of the EP augmenting/reducing measure in predicting lithium response cannot be statistically confirmed in this small sample. However, it is of interest that the strongest augmenter, patient A, was the only child to show both significant global child and global parent rating improvement. The two

patients with significant global parent rating improvement also had higher amplitude/intensity slopes than the three with nonsignificant rating change (Table II). It is interesting that patient B who clinically appeared to respond dramatically was the second highest augmenter.

EP changes with lithium were similar to those observed with lithium in adults - a significant increase in amplitude for the N120 compenent, from placebo to lithium, occipital lead (Oz) for all five children. The average amplitude (across all intensities) went from 9.09 μv to 13.83 μv ($F = 18.08$, 1, 4, n.f., $p < 0.01$). The P100 component at Oz also showed a significant enhancement ($F - 10.20$, $p < 0.05$). This pattern was also maintained in patient B, 12.91 μv baseline, 13.69 μv on lithium.

In conclusion, even our small sample gives further credence to the efficacy of lithium in certain childhood disorders. Our best two responders not only had a BP diagnosis but an electrophysiological pattern, augmenting, which has previously been associated with lithium response. This, taken together with the similarity of the EP changes on lithium to those occurring in adult patients treated with lithium, supports a physiological parallel between bipolar affective illness in adults and children.

Publications:

McKnew, D.H., Cytryn, L., Buchsbaum, M.S., Hamovit, J., Lamour, M., Rapoport, J.L. and Gershon, E.S.: Lithium in children of lithium-responding parents. Psychiatry Research 4: 171-180, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00151-05 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Behavior and Electrophysiological Response of Normal and Hyperactive Children and Normal Adults to a Single Dose of Dextroamphetamine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Christy L. Ludlow, Ph.D.	Speech Pathologist	CDP	NINCDS
	Herbert Weingartner, Ph.D.	Chief, Unit on the Psychobiology of Cognition	LP	NIMH
	Michael H. Ebert, M.D.	Chief, Section on Experimental Therapeutics	LCS	NIMH
	Irwin J. Kopin, M.D.	Chief	LCS	NIMH
	William E. Bunney, Jr.,M.D.	Chief	BP	NIMH

COOPERATING UNITS (if any)
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LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
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☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project has been discontinued

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00152-04 BP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Sinemet on Behavior in Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
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COOPERATING UNITS (if any)

Section on Experimental Therapeutics, LCS, DCBR, NIMH
Laboratory of Clinical Science, DCBR, NIMH, Department of Psychiatry & Pharmacology, USUHS

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Biological Psychiatry Branch

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Unit on Childhood Mental Illness

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NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.40	.25

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A double-blind crossover study evaluated the clinical response of hyperactive boys to Sinemet (maximum dose, 60-600 mg). The behavioral and congitive responses were qualitatively similar to that seen with amphetamine but the effects were much weaker; clinically it does not appear the Sinemet will be useful in the treatment of hyperkinetic children. Theoretically, Sinemet increased urinary MHPG, an index of central norepinephrine metabolism and therefore the mild improvement seen does not support a specific dopamine hypothesis of therapeutic action of stimulants in these children.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Hyperactive children usually respond symptomatically to stimulant medications which are known to increase the concentration of monoamines at the receptor sites in the brain. As L-dopa is known to increase the availability of dopamine in the brain, if a deficiency of dopamine were responsible for at least some cases of hyperkinesis, then L-dopa should be expected to have some therapeutic value.

Methods Employed: The recent practice of combining L-dopa and Carbidopa, a peripheral dopadecarboxylase inhibitor, lowers the necessary dosage and thereby decreases side effects, making this treatment more feasible for children. Hyperactive boys, ages 6-12, exhibiting restlessness and impulsivity both at school and during an inpatient evaluation period were selected for the protocol. Evaluation included medical, neurological, and psychiatric examination as well as psychological testing.

Children were started on a gradually increasing dosage of Sinemet or placebo; medication was increased over the first two weeks to the maximum dose (60-600 mg Sinemet or 6 tablets of placebo), which was given during the third week as tolerated.

During the third week of placebo or Sinemet treatment, plasma norepinephrine and HVA turnover was assessed. Clinical response for the group was compared for drug and placebo periods and individual responses were related to changes in the catecholamine measures.

Major Findings: The project began in June 1978. Eight children received the drug. Because of nausea and vomitting, two children received a maximum dose of 40/400 mg/day of Carbidopa/L-dopa. For the group of eight children completing the study, Sinemet causes a small but significant decrease in hyperactivity on the teacher rating scale. There was a slight decrease in motor activity but this was not significant. There was a trend for improvement on the continuous performance test, a measure of vigilance. There was no improvement on cognitive test performance, in contrast to the highly significant effects seen with amphetamine in similar populations. Plasma NE measured in Dr. Lake's laboratory did not change significantly; urinary MHPG and urinary VMA on the other hand both were significantly elevated. There was no significant correlation between individual improvement scores and changes in urinary MHPG or VMA.

Significance to Mental Health Research: As hyperactivity may be a forerunner of adult sociopathy, alcoholism or schizophrenia, studies on treatment and underlying pathophysiology of hyperactive children have wide application throughout the field of mental health research. Alternate treatments to stimulants are practically useful, as approximately 15% of hyperactive children do not respond favorably to stimulants.

Proposed course of Project: The project has been discontinued.

Publications:

Langer, D., Rapoport, J., Brown, G., Ebert, M., Bunney, W.E., Jr.: Behavioral Effects of Sinemet in Hyperactive Boys. J. Amer. Acad. Child Psychiatry. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00153-04 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Treatment of Obsessional Children and Adolescents with Chlorimipramine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
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COOPERATING UNITS (if any)
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Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.10	.15

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Obsessional disorder of childhood is a rare disorder about which little is known. The purpose of this protocol is to collect basic data on family history of mental illness, sleep measures and neuropsychological testing on a selected group of children and adolescents with obsessional disorder. A drug trial of chlorimipramine, desmethylimipramine, and placebo will be carried out to evaluate the effect of antidepressant and the specificity of chlorimipramine for this disorder.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To examine clinical, family, physiological and neuropsychological measures in childhood Obsessive-Compulsive Disorder of childhood to chlorimipramine and to relate this response to changes in ratings of depression. Clinical response to chlorimipramine will be related to platelet serotonin at baseline.

Methods Employed: Patients are sought on a national level because of the rarity of the disorder. Inclusion criteria are Obsessive-Compulsive Disorder as a primary disturbance. Children must have IQ of 85 or above, and be free from known neurological disturbance or psychosis.

A modification of the Leyton Obsessional Inventory is used to monitor drug effects on Obsessive-Compulsive symptomatology throughout the 12-week clinical trial. Weekly ratings are made by two physicians and ward nurses on the CPRS.

Plasma levels of drug as well as platelet serotonin will be assayed for each drug phase.

Major Findings: Twelve children have entered the protocol: Ten males aged 13-17, and two females aged 13 and 15. The first patient improved gradually throughout his hospital course and did not have a different course during chlorimipramine treatment. Depression ratings changed in parallel with obsessive symptoms. One patient showed dramatic improvement on chlorimipramine and is being maintained on the drug. The others have showed equivocal response.

A problem in interpreting drug effects is the tendency for there to be gradual improvement over the course of the study for almost all of the patients. This group trend obscures individual drug/placebo differences and may mask true drug effects.

Preliminary analysis of family pedigree data is somewhat at variance with other reports of Obsessive-Compulsive Disorders. Only one patient reports obsessive-phobic symptomatology in any family members. The other families have no obsessional illness among first degree relatives. There were a surprising number of siblings who exhibit learning and behavior problems. Six of the 19 siblings age 18 and under have such symptoms. Sleep recordings on the group showed a decrease in REM latency at baseline, which increased over time with clinical improvement. These findings indicate a possible biologic link between depression and obsessive-compulsive disorder in childhood. Speech and language testing suggests mild dysphasia and lack of normal laterality on dichotic listening suggestive of a dysfunction of frontal and/or temporal lobes.

Platelet MAO and 5HT from the patients and a group of matched adolescent controls were not significantly different.

Significance to Mental Health Research: Obsessive-Compulsive Disorder is a rare but extremely disabling condition. About one third of adults with the disorder had their onset during childhood or adolescence. Children with the condition are often very ill and do not respond well to treatment. Relative to other rare conditions such as infantile autism, there has been virtually no research in this area of childhood mental illness.

Proposed Course of Project: A total of 20 patients will be studied. Baseline clinical measures, plasma tricyclic level, and platelet serotonin will be examined in relation to clinical response to the active drugs. Neuropsychological and linguistic testing will be compared for patients and controls.

Publications:

Elkins, R., Rapoport, J. and Lipsky, A.: Obsessive-Compulsive Disorder in childhood: a review. J. Am. Acad. Child Psychiatry 19: 511-524, 1980.

Rapoport, J., Elkins, R., and Lipsky, A.: A pilot trial of Chlorimipramine for adolescents with Obsessive-Compulsive Disorder. Psychopharmacology Bulletin, 16: 61-63, 1980.

Rapoport, J., Elkins, R., Langer, D., Sceery, W., Buchsbaum, M., Gillin, J.C., Murphy, D.L., Zahn, T., Lake, R., Ludlow, C., and Mendelson, W. Childhood Obsessive-Compulsive Disorder. Am. J. Psychiat. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00154-03 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

State Dependent Learning in Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
Other:	Dennis H. Langer, M.D.	Clinical Associate	BP	NIMH
	Herbert Weingartner, Ph.D.	Chief, Unit on the Psychobiology of Cognition	LP	NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, DCBR, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.10	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

State dependent learning has primarily been demonstrated in adults receiving substances which impair learning. However, two studies have raised the possibility that hyperactive children who must learn material while on stimulant drug may manifest state dependency as measured by decreased transfer of learning to the off-drug state.

A total of 15 hyperactive learning disabled children were tested on amphetamine (0.5 mg/kg) and placebo. No evidence for state dependent learning was found.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Hyperactive boys who were at least one year behind age/IQ norms in academic achievement were tested on and off dextroamphetamine (0.5 mg/kg) to see whether verbal material was best retained in the same or different state from which it was learned.

Methods Employed: Fifteen hyperactive boys were tested in four paired learning and retention sessions. Learning and recall was measured during same (drug-drug or placebo-placebo) sessions and different (placebo-drug or drug-placebo) sessions. All sessions were carried out using a double blind, random assignment, counterbalanced order design. Mood scale ratings were also completed for each child for each test session.

Major Findings: A total of fifteen children have been studied to date. There was no support for existence of state dependent learning.

Amphetamine state dependent learning (SDL) was not evident. Amphetamine improved both learning and recall of verbal material. This improvement did not correlate with other measures of clinical improvement.

Significance to Mental Health Research: A major clinical problem in the treatment of hyperactive children is the lack of improvement in academic performance on stimulants in contrast to the clear change in impulsive inattentive behavior. The basis for this remains unknown but it is not likely that state dependency is responsible.

Proposed Course of Project: The study has been completed (July 1981) and the project discontinued.

Publication:

Weingartner, H., Langer, D., Grice, J., Rapoport, J. Acquisition and retrieval of information in amphetamine treated hyperactive children. Submitted J. Abnorm. Psychol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00161-03 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Cognitive Effects of Deitary Substances in Normal and Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
Other:	Alan Zametkin	Clinical Associate	BP	NIMH
	Alan Neims, Ph.D.	Professor of Pharmacology and Pediatrics, U. of Florida, Gainesville, Florida		
	Marvin Cornblath, M.D.	Chief, Pediatric Medicine Branch		NICHD

COOPERATING UNITS (if any)
Laboratory of Psychology and Psychopathology, DCBR, NIMH
Section on Experimental Therapeutics, LCS, DCBR, NIMH; U. of Fla., Gainesville
PMB, NICHD

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.30	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Both caffeine and lecithin, substances within the diet of normal children, have been shown to affect memory and vigilance of normal adults. The effects of these substances will be studied on normal children. Acute and subacute effects of caffeine have been studied. Further studies of carbohydrates are planned.

Project Description:

Objectives: To evaluate the effects of caffeine (4 and 10 mg/kg) in single doses and subacute effects (10 mg/kg/day for 2 weeks) on activity memory and vigilance of normal chidlren.

Methods Employed: Children were paid as normal volunteers for participating in this study. Children were free from psychiatric disorder or cognitive disability.

Double-blind single dose and subacute trials of caffeine were carried out, using cognitive test response as the dependent measures. Side effects will also be evaluated.

Double-blind studies of single doses of caffeine (3 mg/kg and 10 mg/kg) were conducted with 19 grade school boys. In addition, there is an ongoing study of subacute effects of caffeine (10 mg/kg day). In this latter study, subjects had daily caffeine or placebo for two week periods in a double-blind crossover design.

Major Findings:

Acute caffeine study: Single doses of caffeine (10 mg/kg) had several significant effects on the normal children's cognitive and behavioral responses. In contrast to placebo or the lower dose (3 mg/kg), high doses of caffeine increased motor restlessness, while improving vigilance as measured by reaction time and continuous performance test.

The subacute caffeine study with a separate sample of 19 boys, has been completed. During the caffeine period, parents and teachers rate the children as more restless, more nervous and jittery. These findings have implications for the biological basis of anxiety. However, children who were habitual low caffeine consumers were most likely to suffer adverse effects.

Significance to Mental Health Research: There is little known about the influence of diet on normal children's behavior. Caffeine is widespread in our daily diet and the influence of dietary level on both normal and pathological samples has considerable clinical significance. Theoretically, the effects of caffeine resemble mild clinical anxiety. Caffeine may provide a pharmacological model for anxiety in childhood.

Proposed Course of Project: Other dietary substances, particularly sucrose and fructose will be studied using a similar design. However for these studies, hyperactive children with a history suggestive of carbohydrate sensitivity will be studied.

Publications:

Elkins, R., Rapoport, J., Zahn, T., Buchsbaum, M., Weingartner, H., Kopin, I., Langer, D. and Johnson, C. Acute effects of caffeine in normal prepubertal boys. Am. J. Psychiat. 138: 178-183, 1981.

Rapoport, J., Elkins, R., Zahn, T., Buchsbaum, M., Weingartner, H., Kopin, I.: Acute Effects of Caffeine on Normal Prepubertal Boys. In Klein, D.F. and Robkin, J. (Eds.): Anxiety, New Research and Changing Concepts. New York, Raven Press, in press.

Rapoport, J., Elkins, R., Neims, A., Zahn, T., Berg, C. Behavioral and autonomic effects of caffeine in normal boys. Devel. Pharmacol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00162-02 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Treatment of Hyperactive Children with Desmethylimipramine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
Other:	Alan Zametkin	Clinical Associate	BP	NIMH
	Steven Paul, M.D.	Chief, Unit on Pre-Clinical Pharmacology	CB	NIMH
	William Potter, M.D., Ph.D.	Assistant to Chief	CP	NIMH
	Michael Ebert, M.D.	Chief, Section on Experimental Therapeutics	LCS	NIMH

COOPERATING UNITS (if any)

Laboratory of Sleep Physiology, DCBR, NIMH;
Clinical Psychobiology Branch, DCBR, NIMH
LCS

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.40	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A trial of desmethylimipramine is planned to compare the acute and chronic efficacy of this agent in hyperactive children and to relate clinical effects to measures of norepinephrine metabolism.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: As tricyclic antidepressants have been shown to have short term beneficial effects for hyperactive children, this study is designed to examine these effects more closely. DMI acts primarily by blocking reuptake of norepinephrine. By monitoring plasma NE, urinary MHPG and examining clinical effects, changes in NE metabolism and antihyperactive effects could be examined to see if they are related.

Methods Employed: DMI or placebo have been tried in 3-week double-blind study in five children.

Major Findings: Unknown as code has not been broken.

Significance for Mental Health Research: Hyperactivity is a major issue in child psychiatry. Understanding its mechanism may lead to more effective treatment and prevention.

Projected Course of Project: The protocol will continue using DMI and placebo in a double blind noncrossover design. Clinical efficacy of DMI will be examined in relation to chronic (1 month) drug and chemical effects will be compared and related to clinical effects.

Publication:

Rapoport, J., Langer, D., Ebert, M.: Pilot Trial of Mianserin Treatment of Hyperactive Boys. In Greenhill, L. (Ed.): Biological Aspects of Child Psychiatry. New York, Spectrum Publications, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00163-02 BP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Naturalistic Study of Activity Levels of Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	Linda Porrino, Ph.D.	Guest Worker Unit on Childhood Mental Illness	BP	NIMH
Other:	Judith Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
	Thomas Wehr, M.D.	Chief, Clinical Research Unit	CP	NIMH
	David Behar, M.D.	Clinical Associate	BP	NIMH

COOPERATING UNITS (if any)

Clinical Psychobiology Branch, DCBR, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Childhood Mental Illness

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.40	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Because of the new technology for measuring 24 hour activity using the NIH actometor, a study has been designed to examine activity levels of hyperactive children and matched controls at baseline. Following this, activity of hyperactive group will be compared during amphetamine and placebo treatment periods. This is the first study to examine drug effects on motor activity outside of a laboratory setting. Drug effects will be examined in relation to measures of structure of classroom and home environment.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Hyperactive boys and matched controls who are in the same school, grade and classes will be followed for a baseline week to compare 24 hour activity patterns for the groups. Following this, hyperactive boys will be monitored continuously for four weeks. The effects of amphetamine (15 mg) or placebo will be compared in a double-blind ABAB design.

Methods Employed: A total of 20 hyperactive-control pairs will be studied. Activity levels during school, free play and home activities will be compared and related to measures of attention (Continuous Performance Test), and ratings of school and home structure. Children will wear the monitor on belts even when they sleep. Weekly appointments will be kept at which time parent and teacher behavior ratings, side effect, hourly diaries of weekly activities and attentional measures will be obtained.

Major Findings: A total of 12 patient-control pairs have been studied. Preliminary findings indicate that hyperactive children are significantly more restless than controls even during sleep although not markedly so. Drug effects appear biphasic, decreasing activity during the day with some increase in activity in the evening. Whether this represents a "rebound" effect, or simply insomnia secondary to continued direct effect of the drug is not clear.

Significance to Mental Health Research: Only a naturalistic study can relate laboratory findings to clinically relevant situations. The nature of "hyperactivity" is poorly understood. If hyperactive children are truly more restless than their peers it is important to know for what situations this is true. Furthermore, this study will provide data on behavioral "rebound" in the evenings following drug. If this is a major finding, then altered dose schedule would be indicated.

Proposed Course of Project: A total of 20 hyperactive children and their matched controls will be studied. In addition, structured interview of these outpatients in comparison with interviews of "hyperactive" children attending the 3 East day program will be compared to explore the many different diagnostic categories included in the heterogeneous population of "hyperactive" children. Activity patterns from the naturalistic study will be related to diagnostic subgroup.

Publications: None to date.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00164-02 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

A Study of Infants of Parents with Bipolar Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leon Cytryn, M.D.	Medical Officer	BP	NIMH
Other:	Yolande Davenport, M.S.W.	Social Worker	LDP	NIMH
	Martine Lamour, M.D.	Guest Worker	BP	NIMH
	Donald H. McKnew, M.D.	Medical Officer	BP	NIMH
	Marian Yarrow, Ph.D.	Research Psychol.	LDP	NIMH
	Carolyn Waxler, Ph.D.	Research Psychol.	LDP	NIMH
	Ann Barnett, M.D.	Children's Hospital Washington, D.C.		
	Ira Weiss, Ph.D.	Children's Hospital Washington, D.C.		
	Robert Harmon, M.D.	Univ. of Colorado, Denver, Col		
	Theodore J. Gaensbauer, M.D.	Univ. of Colorado, Denver, Col.		
	William E. Bunney, Jr. M.D.	Chief	BP	NIMH
	Frederick Goodwin, M.D.	Chief, Clinical Psychobiology	CPB	NIMH

COOPERATING UNITS (if any)
LDP, NIMH, University of Colorado, Denver, Colorado; Children's Hospital, Washington, D.C.; Clinical Psychobiology Branch, DCBR, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.4	1.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major purpose of this study is to investigate 1) affective states, neuropsychobiological patterns and general development of infants with a parent with bipolar illness; 2) To study the infant-family interaction, particularly the difference between the ill and healthy parent. There is general agreement about the genetic vulnerability of first degree relatives of manic-depressive patients. Such genetic vulnerability may possibly be already evident during infancy. The same may be said about environmental factors, especially the family, causing an affective disorder, which too, may be identified and detected by the study of infant-family interactions.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The major purpose of this study is to investigate 1) affective states, neuropsychobiological patterns and general development of infants with a parent with bipolar illness; 2) To study the infant-family interaction, particularly the difference between the ill and healthy parent. There is general agreement about the genetic vulnerability of first degree relatives of manic-depressive patients. Such genetic vulnerability may possibly be already evident during infancy. The same may be said about environmental factors, especially the family, causing an affective disorder, which too, may be identified and detected by the study of infant-family interactions.

Methods Employed: The development of infants having one parent with a bipolar affective disorder was studied. Two groups were investigated: 1) 6 male infants with one parent who met the RDC criteria for bipolar I illness (3 fathers, and 3 mothers), 2) 6 matched controls, each of whom has both parents free of any psychopathology. Each infant was studied 3 times, at 12-13 months, at 15-16 months, and at 18-19 months. The studies included neurological examination, averaged evoked responses (AER), measurement of affect, Bayley Developmental Scale, temperamental profile, parental rearing attitudes and behaviors, and a detailed family history with emphasis on family psychodynamics.

Major Findings: All the data collection has been completed. Each index and control infant had the following: Neurological examination, developmental examination, average evoked potential, temperamental assessment and three stranger-separation sessions. In addition detailed family assessment and history was obtained. The data are being evaluated. It is anticipated that this evaluation will be completed by late summer or early fall, 1980.

Significance to Biomedical Research and the Program of the Institute: This study may find early behavioral markers of vulnerability to manic-depressive illness. It is, along with the research in the Child Unit on children at risk for later psychiatric disorder, an important area for preventive psychiatry.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00165-01 BP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biological Markers of Alcoholism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
Other:	David Behar, M.D.	Clinical Associate	BP	NIMH
	Marrku Linnoila, M.D.	Guest Worker	BP	NIMH
	Awil Muhkerje	Chief, Section on Molecular and Developmental Genetics	PRB	NICHD

COOPERATING UNITS (if any)

Clinical Psychobiology Branch NIMH
Pregnancy Research Branch NICHD

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Childhood Mental Illness

INSTITUTE AND LOCATION

National Institute of Mental Health, NIH, Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.75	0.75

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of the project is to measure putative biological markers for alcoholism in prepubertal boys who are offspring of alcoholics and in matched controls. Markers include: blood and breath acetaldehyde following a test dose of alcohol and alcohol and acetaldehyde dehydrogenase in skin fibroblasts.

PHS-6040
(Rev. 10-76)

Project Description: Z01 MH 00165-01 BP

Objectives: There is evidence for a genetic factor particularly among male alcoholics. This pilot project will compare blood and breath acetaldehyde for "high risk" and control children.

Methods Employed: Extensive recruitment through area alcohol treatment programs has produced a sample of approximately 15 high risk children and 15 age matched controls. Clinical screening and skin biopsy has been completed. A challenge of alcohol and clinical biological measures will be carried out over the next year.

Major Findings:

None to date

Significance to Mental Health Research: As the treatment of alcoholism has been relatively unsatisfactory in adult samples, the identification and possible prevention of high risk individuals assumes great significance. Alcoholism in adults is a major public health problem.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00176-01 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Treatment of Hyperactive Children with Chlorgyline

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	Judith L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
Other:	Alan Zametkin, M.D.	Clinical Associate	BP	NIMH
	Christy L. Ludlow, Ph.D.	Speech Pathologist	CDP	NINCDS
	Dennis Murphy, M.D.	Chief	CN	NIMH
	Michael H. Ebert, M.D.	Chief, Section on Experi-Mental Therapeutics	BP	NIMH
	William E. Bunney, Jr., M.D.	Chief	BP	NIMH

COOPERATING UNITS (if any)

Section on Experimental Therapeutics	LCS	NIMH
Clinical Neuropharmacology Branch		NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH Bethesda Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.75	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hyperactive boys will be treated with up to 15 mg/day of Clorgyline selective Monoamine Oxidase A inhibitor, or amphetamine (0.5 mg/kg). Behavioral measures include Activity, Vigilance and Parent and Teacher Behavior Ratings. Biological measures include Urinary MHPG, PEA, and Platelet MAO. The aim of the study is to elucidate neurotransmitter mechanism in amphetamines efficacy in hyperactivity.

PHS-6040
(Rev. 10-76)

Project Description: Z01 MH 00176-01 BP

Objectives: To see if an MAO Inbititor is an efficacious alternative to amphetamine treatment of hyperactivity. To elucidate the neurotransmitter mechanisms in amphetamine treatment of hyperactivity.

Methods Employed: Hyperactive boys will be given amphetamine (0.5 mg/kg), placebo, and Clorgyline (up to 15 mg) using a double blind crossover design modified by a 2-week placebo washout period between active drugs. Urinary catecholamine metabolites and platelet MAO will be measured to see if they predict or reflect drug effects.

Major Findings: Three children have completed the 11-week protocol to date. Clinical impression is that Clorgyline is as effective as amphetamine in improved behavior and vigilance. There have been no adverse reactions to Clorgyline.

Significance to Mental Health Research: As hyperactivity may be a forerunner of adult sociopathy, alcoholism and schizophrenia, studies on the treatment and pathophysiology of hyperactive children have wide implications throughout the field of mental health research.

Proposed Course of Project: A total of 20 children will be studied on the protocol.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00034-11 BP

PERIOD COVERED
October 1, 1980 through September 30 1981

TITLE OF PROJECT (80 characters or less)
Psychological and Physiological Correlates of the Average Evoked Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Section on Clinical Psychophysiology	BP NIMH
OTHER:	R. Coppola, D.Sc.	Senior Engineer Laboratory of Psychology	LPP NIMH
	R. A. Lavine	George Washington Univ. Washington, D.C.	Geo. Wash. Univ.

COOPERATING UNITS (if any)
Laboratory of Psychology and Psychopathology
George Washington Univ., Washington, D.C.

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Clinical Psychophysiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Evoked potentials at varying intensities of stimulation are being recorded at 16 points on the left hemisphere and computer interpolated maps being developed.

PHS-6040
(Rev. 10-76)

Project Description:

Evoked potential research has been given emphasis by this Section because evoked potentials show high heritability and sensitivity to attentional, as well as neuropharmacological manipulation--coupled with the possibility of cortical or subcortical localization; these features make it an attractive technique for biological research in psychiatry.

Deficits in attention have been widely reported in both schizophrenia and childhood hyperactivity. These results, more consistent than many neurochemical findings, have led theorists to suggest that the neurochemical and electrophysiological mechanisms of attention may provide a key for understanding these illnesses. In psychiatric patients, many different features of the attentional processes have been reported to be deficient, including selective attention, vigilance and switching attentional sets. Abnormalities of the motor system itself, including elevations in creatinine phosphokinase and pathological muscle fibers have been reported. EMG studies of schizophrenics' reaction time suggest that peripheral factors may be important in differences previously held to be due solely to central dysfunction.

These and other studies have demonstrated the difficulty that psychotic patients have in normally attending to, selecting, and assimilating incoming sensory information. Does this indicate that a specific perceptual or cognitive deficit can be identified, leading ultimately to a precise neurophysiological explanation of the schizophrenia and the affective disorder? One major problem investigators face is that the very same attentional and information processing deficits under study may interfere in rather unpredictable ways with a psychotic patient's performance. It therefore, remains unclear to what extent defective attention, motivation, understanding of instructions, hallucinations or poor muscle coordination are the sources of poor performance on a psychophysical test.

The evoked potential (EP) technique seems particularly appropriate for separating these differing central and peripheral attention factors and for localizing attentional deficits, and in particular cortical areas. By examining the sensory response at a neurophysiological level, using EP techniques, some of the methodological problems may be partially overcome. Tasks requiring little or no instructions on overt performance can be developed and the need for a motor response obliterated.

From perceptual studies, we might expect that measurement of sensory response at a neurophysiological level using average evoked potential techniques would tend to indicate reduced reactivity in schizophrenics and increased reactivity in patients with schizophrenia and bipolar affective disorders. In general, schizophrenics show low amplitude EPs to visual, auditory or somatosensory stimuli.

Differences between schizophrenics and normals in EP amplitude could reflect individual differences in the scalp distribution of the components, rather than merely quantitative reduction. To investigate this, we have developed

a technique for representing EP data as a topographic map. For the cortical maps, in addition to the 12 electrodes normally on the left side (10-20 system) or midline (C_z-p_z-O_z), four additional electrode locations were added to provide greater resolution of the posterior cortex. These are placed at the centers of squares formed by connecting P_3-P_2-O_z-O_1 (PO), C_3-C_z-P_z-P_3 (CP), T_3-C_3-P_3-T_5 (TCP) and F_7-F_3-C_3-T_3 (FTC). The 16 values were then used to interpolate values over the entire scalp area. Studies of schizophrenia are underway, with age and sex-matched controls also being recorded.

Publications:

Buchsbaum, M.S.: Electrical activity of the brain: Age changes and personality differences. In Proceedings of Council Seminars. Durham N.C., Duke University, Center for the Study of Aging and Human Development, Duke University Medical School, (In Press).

Buchsbaum, M.S., Coppola, R., Gershon, E.S., van Kammen, D.P., and Nurnberger, J.I.: Evoked potential measures of attention and psychopathology. Adv. biol. Psychiat., (In Press).

Buchsbaum, M.S. and Johnson, J. L.: Electrophysiological correlates of cognitive dysfunctions in psychiatric patients, including senile dementia, Hungarian Academy of Sciences, Budapest, (In Press).

Lavine, R.A., Buchsbaum, M.S., and Schechter, G.: Human somatosensory evoked response: Effects of attention and distraction on early components Physiological Psychology, 8: 405-408, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00035-09 BP

PERIOD COVERED October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biochemical and Psychopharmacological Correlates of the Average Evoked Response in Psychiatric Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum	Chief, Section on Clinical Psychophysiology	BP NIMH
OTHER:	F. K. Goodwin	Chief,	CP NIMH
	D. L. Murphy	Chief,	CN NIMH
	T. P. Zahn	Research Psychologist	LPP NIMH
	R.D. Coursey	University of Maryland	Dept. Psychiatry
	R.J. Haier	Brown University	Dept. Psychiatry
	I.I. Gottesman	University of Minnesota	Dept. Psychiatry
	Zuckerman, M.	University of Delaware	Dept. Psychiatry

COOPERATING UNITS (if any)

Clinical Psychobiology Branch; Clinical Neuropharmacology Branch; Laboratory of Psychology and Psychopathology; DCBR, NIMH. Department of Clinical Psychology, University of Maryland, Department of Psychiatry University of Minnesota

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Clinical Psychophysiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	0.6	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Evoked responses (EPs), platelet MAO, and urinary MHPG were studied in normal volunteers and patients with affective disorders. Relationships between EPs and these measures revealed important individual differences.

PHS-6040
(Rev. 10-76)

Cooperating Units (Continued)

Department of Psychiatry, Brown University, Providence, Rhode Island.

Project Description

This year we reviewed the trait of sensation-seeking and the biological phenomena associated with it: strength of the initial orienting reflex, augmenting versus reducing of the average evoked potential, the enzyme monoamine oxidase, and gonadal hormones (both androgen and estrogens). Both the psychological test and biological measures have retest reliabilities that are characteristic of stable traits. Twin studies show moderate to strong genetic control of the personality and of several of the biological measures. Several behavioral characteristics that are associated with the trait in humans are also related to the biological traits in animals, including sociability and social dominance, reactivity to novel situations, and play. In humans, sexual experience, interest in new situations, experiences with drugs, manic-depressive tendencies, and psychopathy are associated with both sensation-seeking and its biological correlates. Age and sex-differences show some parallels and some differences between sensation-seeking and its biological correlates. The convergence of findings points up some new directions for research in the role of central monoamines and their regulators in the development of basic personality traits.

Somatosensory EP studies were also studied in patients with affective disorders. Stressful procedures are reported to increase urinary MHPG in both normal and depressed patients. Bipolar depressed patients have also been shown to be especially pain tolerant in comparison to normals. In the present study, 12 depressed patients (6 bipolar and 6 unipolar patients) and 10 normal volunteers had average evoked potentials recorded for visual and painful electrical stimuli. MHPG urinary excretion was measured during this session and during an unstimulated resting session on the home ward. Male, normal volunteers showed a significant increase in urinary MHPG under stress, while MHPG increment was noted for the depressed patient group. Depth of depression, assessed by the Zung and Beck scales, was found to be correlated with the reduced urinary MHPG response to stress.

Publications

Buchsbaum, M.S. and Ludwig, A.M.: Effects of sensory input and alcohol administration on visual evoked potentials in normal subjects and alcoholics. In Begleiter, H. (Eds.): Biological Effects of Alcohol. New York, Plenum, 1980, pp. 561-571.

Coursey, R.D., Buchsbaum, M.S. and Murphy, D.L.: Psychological characteristics of subjects identified by platelet MAO activity and evoked potentials as biologically at risk for psychopathology. J. Abn. Psychology. 89(2): 151-164, 1980.

Coursey, R.D. and Buchsbuam, M.S.: The biological high risk research strategy: A review of recent studies. In Regier, D. A. and Allen, G. (Eds.): Risk Factor Research in the Major Mental Disorders. National Institute of Mental Health, DHHS Publ. No. (ADM)81-1068, Washington, D.C.: Supt. of Docs., U.S. Government Printing Office (In Press).

Coursey, R.D., Buchsbaum, M.S. and Murphy, D.L.: Monoamine oxidase activity and the longitudinal biological high risk approach to schizophrenia and affective illnessses. In Mednick, S.A. and Harway, M. (Eds.): Longitudinal Research in the United States. Boston, Martinus Mijhoff, (In Press).

Zuckerman, M., Buchsbaum, M.S. and Murphy, D.L.: Sensation-seeking and its biological correlates. Psychological Bulletin. 88: 187-214, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00036-07 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Individual Differences in Sleep and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Section on Clinical Psychophysiology	BP	NIMH
OTHER:	J. C. Gillin, M.D.	Research Psychiatrist	BP	NIMH
	R. M. Post, M.D.	Chief, Section on Psychobiology	BP	NIMH
	W. B. Mendelson, M.D.	Research Psychiatrist	SMR	NIMH

COOPERATING UNITS (if any)

Clinical Psychopharmacology, St. Elizabeth's Hospital, Washington, D.C.

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Clinical Psychophysiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

New techniques for analyzing local EEG processes during sleep stages are under development.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

The effects of a single night of sleep deprivation on the amplitude and amplitude/stimulus intensity function of the visual evoked potential (EP) were studied in 16 patients with primary affective disorder and 20 normal controls. Visual EP amplitude and amplitude/intensity slopes tended to increase after sleep deprivation in the patient group, a direction found in other studies to be associated with a spontaneous mood improvement and changes with antidepressant medication. In contrast, normal volunteers who showed little or no mood elevation showed generally opposite EP effects. Taken together, the results suggest a similarity in the neurophysiological effect of sleep deprivation between patient and antidepressant medication in depressed patients.

PUBLICATIONS:

Buchsbaum, M.S., Gerner, R. and Post, R.M.: The effects of sleep deprivation on average evoked responses in depressed patients and in normals. Biological Psychiatry. 16: 351-363, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00037-09 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Genetic Factors in Psychiatric Illness and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Section on Clinical Psychophysiology	BP NIMH
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	J. I. Nurnberger	Sect. on Psychogenetics	
	R. Cromwell, M.D.	University of Rochester	
	R. D. Coursey, M.D.	University of Maryland	
	L. B. Purchall, M.D.	University of Maryland	
	X. O. Breakefield Ph.D.	Associate Professor	Yale University
	C. M. Castiglione, M.S.	Associate in Research	Yale University
	E. L. Giller, Jr., M.D.	Associate Professor	Yale University

COOPERATING UNITS (if any)
University of Rochester, Rochester, New York
University of Maryland, College Park, Maryland
Yale University

LAB/BRANCH
Biological Psychiatry Branch

SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Auditory and visual average evoked potentials (EPs) are being studied in a sample of identical twins given a placebo-controlled amphetamine trial. Genetic, hemispheric lateralization and reliability of drug effects will be assessed.

Research strategies in biological psychiatry were reviewed with emphasis on genetic approaches and methodological problems in group comparisons.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

The role of heredity in psychiatric illness is supported by the many studies demonstrating excessively high rates of illness in persons with a genetic relationship to known patients. The clinically variable expression of psychiatric disorders and the difficulty in identification of mildly affected relatives point to the need for biological markers for the genetic vulnerability. The heritability of AER measures combined with their applicability to both ill and well relatives makes them a promising approach. Further, differences have been demonstrated between patients with schizophrenia and various types of affective disorders.

A new collaborative study with Dr. Cromwell of the University of Rochester has been initiated. This project has the assumption that deficits (and other variants) linked to schizophrenic vulnerability may be more useful than global clinical diagnostic variables in investigating genetic factors associated with that disorder. These vulnerability-linked variants tend to be identifiable (1) before, during, and after active episodes of schizophrenic illness, (2) in healthy first-degree relatives, and/or (3) in risk populations with personality traits suggestive of later schizophrenic breakdown. Another assumption is that better genetic understandings of schizophrenia may be obtained from the study of extended families both with (a) multiple and (b) single known instances of schizophrenia together with (c) a pathology-free control group.

Large pedigree families will be examined with focus primarily upon five schizophrenia-related deficits. Of these, two are behavioral (reaction time cross-over, span of apprehension with forced-choice/partial-report procedure), two are biochemical (blood platelet monoamine oxidase and plasma dopamine-beta-hydroxylase), and one is electro-physiological (visual evoked response augmenting/reducing). Each measure will first be analyzed independently and blindly of clinical symptoms of the index family member. Wherever genetic segregation patterns are established for a variant, linkage studies will be conducted. In addition to the genetics analyses, intercorrelation and group comparison of the deficit variables in the experimental groups will be made. Finally, the subjects at extremes on the five experimental variables will be examined blindly on clinical symptom variables (current and historic) and for clinical diagnosis.

No differences in levels of type A monoamine oxidase (MAO) were observed in cultured human skin fibroblasts from 9 patients with bipolar depressive illness as compared to 18 age, sex, and racematched controls. All cells were biopsied and cultured under parallel conditions. Fibroblasts from monozygotic twins (three sets) had levels of MAO activity that were highly concordant, indicating that levels measured in fibroblasts are genetically determined. Together these findings suggest that an inherited predisposition to bipolar depressive illness does not involve inherited variation in levels of type A MAO activity. Using a larger control population, a positive correlation was observed between age of donor and level of MAO activity.

This finding demonstrates the need for age-matched control and patient groups when comparing levels of type A MAO in fibroblasts.

PUBLICATIONS:

Breakefield, X.O., Giller, E.L., Jr., Nurnberger, J.I., Jr., Castiglione, C.M., Buchsbaum, M.S., and Gershon, E.S.: Monoamine oxidase type A in fibroblasts from patients with bipolar depressive illness. Psychiatry Research. 2: 307-314, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00038-07 BP

PERIOD COVERED: October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Laterality, Psychopathology and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Section on Clinical Psychophysiology	BP NIMH
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	V. Reus, M.D.	Staff Psychiatrist	BP NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Clinical Psychophysiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00039-07 BP

PERIOD COVERED: October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Sensory Thresholds and Averaged Evoked Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	G. C. Davis, M.D.	Staff Psychiatrist	University of Tennessee
	F. K. Goodwin, M.D.	Chief, Clinical Psychobiology Branch	CP NIMH
	R. M. Post, M.D.	Chief, Section on Psychobiology	BP NIMH
	D. L. Murphy, M.D.	Chief, Clinical Neuropharmacology Branch	CNB NIMH
	M. Webster	Research Assistant	CP NIMH

COOPERATING UNITS (if any)

Clinical Neuropharmacology Branch; Psychobiology Branch, DCBR, NIMH.
Department of Psychiatry, University of Tennessee, Memphis, Tennessee.

LAB/BRANCH: Biological Psychiatry Branch

SECTION: Clinical Psychophysiology

INSTITUTE AND LOCATION: NIMH, ADMAHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.3	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Average evoked responses (AER) to varying intensities of stimulation to the forearm and subjective ratings of these stimuli have been studied in 155 age and sex matched normal volunteers. The effect of analgesics in normals and naltrexone in schizophrenics was also studied.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Pain and discomfort have long been known as the constant companions of psychiatric illness. Do patients with major affective illness and schizophrenia have a disturbed perceptual experience of pain as a result of their illness? What are the effects of major psychotropic drugs on pain? Are psychotropic drugs analgesics? In this era of neuropsychopharmacology, these questions can be more effectively approached than before. Many of the neuronal and behavioral actions of these drugs are now known and, in addition, we are beginning to understand the neuronal mechanisms for integration of pain. Thus, particular emphasis on the psychological aspects of pain in future research is desirable.

A new electric stimulation pain assessment technique using the Tursky electrode and a non-parametric analysis of subjects ratings was found sensitive to aspirin, morphine and opiate antagonists in a series of double-blind cross-over trials in normal adults. Stable individual differences in pain sensitivity (off-medication) were maintained on two testing sessions seven months apart. Older individuals were less pain sensitive than younger individuals. Men were more insensitive than women under age 30. Together, these results suggest the empiric usefulness of this pain measurement technique.

Somatosensory evoked potentials to stimuli ranging from barely perceptible to painful were recorded in 153 normal adults. Reliability of amplitude and amplitude/intensity slopes was demonstrated in 29 individuals tested twice, two or more weeks apart. In randomized, double-blind, placebo controlled trials, both aspirin and morphine significantly diminished the N120 component at high stimulus intensities. Age, sex, and pharmacological effects paralleled those found with psychophysical techniques described above.

Somatosensory and visual evoked potentials were recorded in 15 normal volunteers given single doses of either apomorphine (0.75 mg, subcutaneously), haloperidol (1 mg, i.m.), or saline placebo (i.m.) on three separate days. Apomorphine and haloperidol reduced EP amplitude for both somatosensory and visual EPs. The EP latency in the somatosensory system was significantly shortened by apomorphine and lengthened by haloperidol; similar trends were seen for visual EPs. These findings with dopamine agonists and anatgonists suggest the sensitivity of EPs either for the assessment of dopamine mechanisms or for some secondary effect of these drugs, such as one on the endorphin system.

PUBLICATIONS:

Davis, G. C., Buchsbaum, M.S., and Bunney, W.E., Jr.: Pain and psychiatric illness. In Ng, L. and Bonica, J. (Eds.): Pain, Discomfort and Humanitarian Care. Amsterdam, Elsevier North Holland, Inc., 1980, pp. 221-231.

Davis, G. C., Buchsbaum, M.S., and Bunney, W.E., Jr.: Endorphins: Endogenous

control of the perception of pain. In Wain, H. and DeVanis, D. (Eds.): Current Concepts in Pain. Springfield, Illinois, Charles Thomas (in press).

Buchsbaum, M.S., Davis, G.C., and van Kammen, D.P.: Diagnostic classification and the endorphin hypothesis of schizophrenia: Individual differences and psychopharmacological strategies. In Baxter, C.F. and Melnechuk, T. (Eds.): Perspectives in Schizophrenia Research. New York, Raven Press, 1980, pp. 171-191.

Davis, G.C., Buchsbaum, M.S., and Bunney, W.E., Jr.: Alterations of evoked potentials link research in attention dysfunction to peptide response symptoms of schizophrenia. In Costa, E. and Trabucchi, M. (Eds.): Neural Peptides and Neuronal Communications. New York, Raven Press, 1980, pp. 473-487.

Buchsbaum, M.S, Davis, G.C., Goodwin, F.K,. Murphy, D.L., and Post, R.M.: Psychophysical pain judgments and somatosensory evoked potentials in patients with affective illness and normal adults. In Perris, C., and von Knorring, L. (Eds.): Recent Advances in Biological Psychiatry. Basel, Switzerland, S. Karger, 1980, pp. 63-72.

Davis, G.C., Buchsbaum, M.S., Naber, D., and van Kammen, D.P.: Effect of opiates and opiate antagonists on somatosensory evoked potentials in patients with schizophrenia and normal adults. In Perris, C., von Knorring, L., and Kemali, D. (Eds.): Clinical Neurophysiological Aspects of Psychopathological Conditions. Basel, Switzerland, S. Karger, 1980, pp. 73-80.

Davis, G.C., Buchsbaum, M.S. and Bunney, W.E., Jr.: Opiates, opioid peptides and psychiatry. Annals New York Academy of Science, (In Press).

Naber, D., Davis, G.C., Buchsbaum, M.S. and Pert, C.B.: Effects of morphine and naloxone on pain sensitivity and somatosensory evoked potentials/relationships to B-endorphin plasma level. Arzneim.-Forsch./Drug Res., 30(II): 1236, 1980.

Uhde, T.W., Post, R.M., Siever, L.J. and Buchsbaum, M.S.: Clonidine and psychophysical pain. The Lancet, II: 1236, 1980.

Buchsbaum, M.S., Davis, G.C., Coppola, R., and Naber, D.: Opiate pharmacology and individual differences: I. Psychophysical pain measurements. Pain (In Press).

Buchsbaum, M.S., Davis, G.C., Coppola, R. and Naber, D.: Opiate pharmacology and individual differences: II. Somatosensory evoked potentials. Pain, (In Press).

Rey, A.C., Buchsbaum, M.S. and Post, R. M.: Apomorphine, haloperidol and the average evoked potentials in normal human volunteers. Communications in Psychopharmacology (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00040-06 BP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Amphetamine Pharmacokinetics and the Average Evoked Responses in Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Section on Clinical Psychophysiology	BP	NIMH
	M. H. Ebert, M.D.	Chief, Section on Experimental Therapeutics	LCS	NIMH
	G. L. Brown, M.D.	Medical Officer, Unit on Childhood Mental Illness	BP	NIMH
	J. L. Rapoport, M.D.	Chief, Unit on Childhood Mental Illness	BP	NIMH
	A. J. Sostek, Ph.D.	St. Elizabeth's Hospital		NIMH

COOPERATING UNITS (if any)
Laboratory of Clinical Studies, DCBR, NIMH
St. Elizabeth's Hospital

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Clinical Psychophysiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of amphetamine on the average evoked response (AER) is being studied in hyperactive children and normal adults.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

An adaptive-rate continuous performance task for the assessment of human performance has been developed based on the original method of Rosvold and Mirsky. The stimulus presentation speeds up after correct detections and slows down after errors of omission or commission, allowing applications in a wide range of clinical populations and normal age groups. Adequate error rates are obtained to characterize each individual's performance. Signal detection analysis yields d' and beta parameters with adequate test-retest reliability over 6 months. A specific decline in d' over time is consistent with other studies of human vigilance.

Deficient sustained attention is a symptom of hyperactivity that can be improved by stimulant medication. Recently, amphetamine has been shown to increase detections during a vigilance task in both normal and hyperactive boys. The present study applied signal detection analysis to the vigilance performance of 15 hyperactive and 14 normal boys divided into two age groups (6-9 and 10-12). A computerized continuous performance test was administered under amphetamine and placebo. Overall group comparisons indicated that perceptual sensitivity or d' was higher for the normal boys and the older groups, and analysis of drug treatments showed that amphetamine significantly increased d'. Interactions between drugs and age groups demonstrated that amphetamine affected the younger boys to a significantly greater degree than the older children for both d' and response bias or beta. It is notable that the results were essentially parallel for both normal and hyperactive children.

Six offspring of manic-depressive patients, whose parents were lithium responders, were selected on the basis of their incapacitating psychopathology for treatment with lithium. The children ranged in age from 6 to 12. A double-blind, crossover design was used over 16-18 weeks. Weekly ratings were done, and average evoked potential (EPs) were measured at each crossover. Two children diagnosed as having a bipolar affective disorder had a clear-cut response to lithium and were strong augmenters on the EP. This, taken together with the similarity of the EP changes on lithium to those occurring in adult patients treated with lithium, supports a physiological parallel between bipolar affective illness in adults and children.

PUBLICATIONS:

Rapoport, J.L., Buchsbaum, M.S., Weingartner, H., Zahn, T.P., Ludlow, C., Bartko, J., Mikkelsen, E.J., Langer, D.H., and Bunney, W.E., Jr.: Dextro-amphetamine: Cognitive and behavioral effects in normal and hyperactive boys and normal adult males. Archives of General Psychiatry. 37: 933-943, 1980.

Weingartner, H., Rapoport, J.L., Buchsbaum, M.S., Bunney, W.E., Jr., Ebert, M.H., Mikkelsen, E.J., and Caine, E.D. Cognitive processes

in normal and hyperactive children and their response to amphetamine treatment. Journal of Abnormal Psychology. 89: 25-37, 1980.

Rapoport, J.L., Buchsbaum, M.S., Cain, R.B. and Vannier, M.W.: Similarity of effects of alcohol and sensory stimulation in post-alcoholics and controls. Alcoholism (NY). 3: 248-251, 1979.

Sostek, A.J., Buchsbaum, M.S., Rapoport, J.L.: Effects of amphetamine on vigilance performance in normal and hyperactive children. Journal of Abnormal Child Psychology. 8: 491-500, 1981.

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Buchsbaum, M.S. and Sostek, A.J.: An adaptive-rate continuous performance test: Vigilance characteristics and reliability for 400 male students. Perceptual and Motor Skills. 51: 707-713, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00041-01 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Simultaneous Electroencephalography and Cerebral Glucography with Positron Emission Tomography (PET) in Normals and Patients with Schizophrenia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHERS:	R. Kessler, M.D.	Staff Physician	CC NIH
	W. E. Bunney, Jr., M.D.	Chief, Biological Psychiatry Branch	BP NIMH
	J. Cappelletti	Computer Specialist, Sect. on Clin. Psychophysiology	BP NIMH
	R. Coppola, D.Sc.	Senior Engineer	LPP NIMH
	D. P van Kammen, M.D.	Chief, Sect. on Neurophyschopharmac.	BP NIMH
	F. Rigal, M.D.	Visiting Scientist	BP NIMH
	R. Waters, M.D.	Clinical Associate	BP NIMH
	L. Sokoloff, M.D.	Chief, Lab. of Cerebral Metabolism	LCM NIMH
	D. Ingvar, M.D.	Visiting Scientist	Univ. of Lund, Sweden

COOPERATING UNITS (if any)
Clinical Center, NIH; LPP, NIMH; LCM, NIMH; and University of Lund, Sweden.

LAB/BRANCH
Biological Psychiatry Branch

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3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have simultaneously investigated local glucose metabolism using 18F-2DG with (PET) and EEG frequency with 16 lead topographic mapping in eight unmedicated schizophrenics and six, age-sex matched, normal controls. Preliminary analysis indicate that most schizophrenics show higher 2DG uptake in occipital cortex than frontal cortex, with clear delineation of occipital gray matter. In contrast, this is reversed in normals, with a frontal maximum and occipital gray less elevated. Results of these parallel studies indicate similarities in distribution for certain EEG frequencies.

PHS-6040
(Rev. 10-76)

Project Description

Studies of cerebral blood flow in man and animal studies using autoradiographs of [C14]-2-deoxyglucose (2DG) have indicated that local cerebral glucose use may parallel local functional activity and that blood flow and EEG frequency measures may be correlated. We have simultaneously investigated local glucose metabolism using 18F-2DG with (PET) and EEG frequency with 16 lead topographic mapping in eight unmedicated schizophrenics and six, age-and-sex matched, normal controls. Subjects sat in an acoustically treated darkened room with eyes closed for ten minutes prior to, and thirty minutes following injection of 3 to 5 mCi 18F-2DG. Following uptake, seven to eight horizontal scans parallel to the CM line were made. Glucose uptake was expressed in micromoles glucose/100g tissue/min following equation in Sokoloff. EEG recordings are made beginning one minute after injection of the isotope and continuing for thirty minutes using 12 standard 10/20 system points on the left hemisphere and midline, and four additional points between existing posterior leads. Ten-second EEG epochs are edited for artifacts and then analyzed using fast Fourier transform techniques. Using a cross-sectional whole head atlas, a standardized, approximately equal area 2D representation of a lateral view of the brain was developed and 10/20 system scalp coordinates projected onto it. EEG power estimates are interpolated for all points on this brain map.

Using digital techniques, consecutive PET slices have the skull and skin layers peeled off by adapting a technique. Next, a 1 cm thick cortical strip is peeled off the slice, conformed to the lateral brain view and values between strips interpolated. The result is two simultaneously obtained electrophysiological and metabolic lateral views of brain function displayed in gray scale values represented by dot density.

Preliminary analysis indicates that most schizophrenics show higher 2DG uptake in occipital cortex than frontal cortex, with clear delineation of occipital gray matter. In contrast, this is reversed in normals, with a frontal maximum and occipital gray less elevated. These preliminary results are consistent with the blood flow studies of Ingvar and the PET results of Farkas et al.

Correlations between specific symptoms and 18F-2DG uptake patterns have been noted. For example, one patient who had auditory hallucinations during the thirty minute uptake period showed elevated glucose use in the superior temporal auditory areas in the left, but not right temporal cortex.

Results of these parallel studies indicates similarities in distribution for certain EEG frequencies. Individual differences in distribution and statistical problems in comparing complex topographic distributions will be discussed. These findings are clearly preliminary and issues of control of mental activity, structure identification, and biological heterogeneity of schizophrenia remain to be explored.

PUBLICATIONS:

Buchsbaum, M.S., Kessler, R., Bunney, W.E., Jr., Cappelletti, J., Coppola, R., van Kammen, D.P., Rigal, F., Waters, R., Sokoloff, L. and Ingvar, D.: Simultaneous electroencephalography and cerebral glucography with positron emission tomography (PET) in normals and patients with schizophrenia. In Tenth International Symposium on Cerebral Blood Flow and Metabolism. St. Louis, Missouri, Washington University School of Medicine, (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00111-07 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Drug Abuse Studies/Stimulant Abuse/Opiate Abuse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.		
William E. Bunney, Jr., M.D.	Chief	BP, NIMH
David Pickar, M.D.	Staff Psychiatrist, Unit on Studies of Drug Abuse	BP, NIMH
Martin R. Cohen, M.D.	Guest Worker	BP, NIMH
Other:		
Elliot S. Gershon, M.D.	Chief, Section on Psychogenetics	BP, NIMH
Dieter Naber, M.D.	Guest Worker	BP, NIMH
Irl Extein, M.D.	Guest Worker	BP, NIMH
John Nurnburger, M.D.	Staff Psychiatrist, Section on Psychogenetics	BP, NIMH
S.C. Schulz, M.D.	Guest Worker	BP, NIMH

COOPERATING UNITS (if any)

Clinical Psychobiology Branch, DCBR, NIMH

Section on Psychogenetics

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Studies of Drug Abuse, BPB, NIMH

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	1.0	1.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project studies the biochemistry of mood alterations induced by drugs of abuse. Several drugs are under investigation: amphetamine, ethanol and opiates. Research strategies include the measurement of opioid activity in plasma during acute drug administration and during withdrawal states. Further research strategies are currently under evaluation.

PHS-6040
(Rev. 10-76)

Names, Laboratory and Institute Affiliations Continued:

Daniel P. van Kammen	Section on Neuropsychopharmacology	BP, NIMH

Objectives

A major effect of drugs of abuse is the production of alterations in mood. While drugs such as ethanol, amphetamine and opiates differ pharmacologically, they share a common clinical effect: the production of an enhanced state of well being. This "high" is a major target of our investigation. Since opiate alkaloids are thought to produce their behavioral and biologic effects through binding to the same opiate receptor as do the naturally occuring opioid peptides (endorphins), it is hypothesized that endorphins may mediate other drug-induced states. To date, the phenomena of dependence, tolerance and withdrawal, well known aspects of opiate pharmacology, have not been directly related to the endorphin system. Recent reports have suggested that the psychomotor depressant effects of ethanol can be blocked by naloxone administration, suggesting possible relationships between ethanol intoxication and endogenous opioids. The activation effect of amphetamine in laboratory animals has been blocked by naloxone. The interrelationships among amphetamine, dopamine, psychopathology and endorphins are currently under investigation.

Methods Employed

1. In collaboration with the Section on Psychogenetics and the Section on Neuropsychopharmacology, the effects of acute amphetamine administration on plasma levels of endogenous opioids have been studied.

2. We have studied the effects of acute ethanol administration on plasma level of endogenous opioids using radioreceptor assay techniques.

3. In collaboration with Dr. Irl Extein of Fair Oaks Hospital, we are studying plasma levels of opioid activity in addicts prior to and during methadone withdrawal. We are examining the effects of the non-opiate drug, clonidine, which is useful in opiate detoxification, on plasma levels of opioid activity.

Major Findings

1. We have found that intravenous amphetamine in normal controls significantly elevates levels of plasma beta-endorphin immunoreactivity and that such changes are significantly correlated with changes in plasma cortisol. We did not find a relationship between behavioral effects of amphetamine and these changes in beta-endorphin. These data differ from those found in the study of intravenous amphetamine in comparable doses in schizophrenic patients. In these subjects, intravenous amphetamine did not significantly elevate plasma beta-endorphin immunoreactivity.

2. The results from a pilot study of ethanol administration in four normal volunteers revealed that intoxicating amounts of ingested ethanol produced significant increases in plasma opioid activity.

3. Levels of plasma beta-endorphin immunoreactivity in opiate addicts maintained on methadone prior to withdrawal did not significantly differ from normal controls. On naltrexone following withdrawal, however, plasma beta-endorphin immunoreactivity was significantly elevated from normal controls. Treatment with clonidine did not produce significant changes in plasma beta-endorphin immunoreactivity.

Significance to Biomedical Research

1. The differential response between normals and schizophrenics, with regard to plasma beta-endorphin immunoreactivity following amphetamine administration, is potentially an important finding. The lack of correlation between changes in beta-endorphin immunoreactivity and behavioral effects of amphetamine, however, raise doubts regarding an endorphin mode of action of amphetamine.

2. The increased levels of plasma opioid activity following ethanol administration in this pilot study is consistent with data suggesting that endogenous opioid system blockade by naloxone reduces motor and cognitive effects of ethanol intoxication. This relationship between ethanol and endogenous opioids is potentially of great importance.

3. The lack of changes in plasma beta-endorphin immunoreactivity among addicted subjects is consistent with animal data in which no changes in opioid ligands or opiate receptors were found in addicted animals. Further research in this area, in particular with oral narcotic antagonist treatment, is indicated.

Proposed Course

1. Systematic study in larger numbers of subjects with regard to behavioral effects of amphetamine and plasma level opioids is proposed. The observed differential response between normals and schizophrenics will also continue to be investigated.

2. The use of naloxone in the systematic study of the effects of ethanol ingestion is proposed.

3. Continued studies of addict populations with particular emphasis on opiate withdrawal are proposed.

Publications

Naber, D., Soble, M., Pickar, D.: Ethanol Increases Opioid Activity in Plasma of Normal Volunteers. Pharmakopsychiatr. Neuropsychopharmacol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00112-04 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Endorphin Research in Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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William E. Bunney, Jr., M.D.	Chief	BP, NIMH

Other:

Robert M. Post, M.D.	Chief, Section on Psychobiology	BP, NIMH
Daniel P. van Kammen, M.D.	Unit Chief, Section on Neuropsychopharmacology	BP, NIMH
Dieter Naber, M.D.	Guest Worker	BP, NIMH
David Rubinow, M.D.	Staff Psychiatrist, Section on Psychobiology	BP, NIMH

COOPERATING UNITS (if any)

Clinical Psychobiology Branch, DCBR, NIMH
Division of Special Mental Health Research, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Studies of Drug Abuse

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.75	1.0	1.75

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Since the discovery of the opiate receptor and endogenous opioid ligands (endorphins), there has been considerable speculation regarding the role of this endogenous opioid system in behavior and mental illness. The major goal of this project is to use available clinical research strategies for the study of endorphins in human behavior and psychiatric illness. The Unit has tested the behavioral effects of intravenously administered naloxone, a pure narcotic antagonist, and of beta-endorphin, an endogenous opioid peptide, in both affectively ill and schizophrenic patients. A sensitive radioreceptor assay, used to determine opioid activity in human CSF and plasma, has been developed and used in conjunction with radioimmunoassay for beta-endorphin to study endogenous opioid function in medication-free and drug treated psychiatric patients.

PHS-6040
(Rev. 10-76)

Names, Laboratory and Institute Affiliations Continued:

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Glenn C. Davis, M.D.	Guest Worker	BP, NIMH
S.C. Schulz, M.D.	Guest Worker	BP, NIMH
Martin R. Cohen, M.D.	Guest Worker	BP, NIMH
Robert M. Cohen, M.D., Ph.D.	Staff Psychiatrist	CN, NIMH
Fredrick K. Goodwin, M.D.	Chief	CP, NIMH
Richard Wagner, M.D.	Staff Psychiatrist, Laboratory of Clinical Psychopharmacology	DSMR, NIMH
Walter Kaye, M.D.	Staff Psychiatrist	LCS, NIMH

Cooperating Units Continued:

Section on Psychobiology, BP, DCBR, NIMH
Section on Neuropsychopharmacology, BP, DCBR, NIMH

Objectives:

The major objective of this project is to seek understanding of the role of endorphins in behavior and mental illness.

Methods Employed:

The following strategies have been used to study the role of endorphins in psychiatric patients.

1. Narcotic antagonist treatment. Depressed and schizophrenic patients from the Clinical Center and St. Elizabeth's Hospital have been administered with double-blind methodology the narcotic antagonist, naloxone, accompanied by careful clinical ratings to delineate possible behavioral effects of this drug. The Unit has served as the coordinating center for a World Health Organization Collaborative Project studying the effects of naloxone in schizophrenia and mania. Naloxone has also been administered to normal volunteers to study biological effects of endogenous opioid system blockade.

2. Administration of opiate alkaloids. In collaboration with the Clinical Psychobiology Branch and Nursing Unit 4-West, we have administered intravenously methadone and placebo to depressed patients to study the acute behavioral effects of opiates in affective illness and to study the neuroendocrine responses to opiates in these subjects. In addition, we have begun oral trials of methadone to test antidepressant efficacy.

3. Beta-endorphin administration. In collaboration with Nursing Units 4-West, 4-East and St. Elizabeth's Hospital, we have completed a pilot double-blind study of the behavioral and biologic effects of acute beta-endorphin administration in psychiatric patients.

and ACTH. Furthermore, it supports a need for careful control for time of CSF sampling in clinical studies.

CSF opioid activity was determined in 89 medication-free psychiatric patients and in 41 medication-free normal volunteers studied under controlled conditions on three nursing units (3-West, 4-West and 4-East). A significant decrease in opioid activity was found among schizophrenics as a whole compared to normals, although this difference was attributed to a rather marked deficit in opioid activity by the male schizophrenics in comparison with male controls; female schizophrenics were found to have comparable CSF opioid activity to female controls; No diffferences in CSF opioid activity were found between depressed subjects and normals including analysis by sex. In a subgroup of these patients, beta-endorphin immunoreactivity was determined. There were no differences in CSF beta-endorphin immunoreactivity between any diagnostic group and normal controls, including sex analysis, suggesting that the observed deficit in CSF opioid activity between male schizophrenics and male normals was related to opioid(s) other than beta-endorphin.

CSF opioid activity in the depressed patients, but not in normals, was significantly correlated with urinary free cortisol excretion. These data suggest that the observed activation of the hypothalamic-pituitary-adrenal axis in depression may be related to opioid activity. In a subgroup of depressed patients who were treated with the anticonvulsant and antidepressant drug, carbamazepine, pre-treatment CSF opioid activity significantly predicted response to drug treatment.

CSF samples from six patients with anorexia nervousa were assayed for opioid activity when patients were at minimal weight and at maximal weight following refeeding. CSF opioid activity was significantly elevated during the period of minimal weight in comparison to maximal weight; this opioid activity at minimum weight was significantly greater than that found in normal female controls, while subsequent levels, when the subjects were refed, were comparable to normal levels.

Significance to Biomedical Research:

1. Results from the World Health Organization Collaborative Project are relevant to research in endorphins and schizophrenia. The finding that concurrent neuroleptic treatment was associated with a positive response to naloxone suggests a possibility of a synergistic effect between chronic dopamine blockade by neuroleptics and acute endorphin blockade by naloxone. Such a relationship between the endogenous opioid system and dopamine system is consistent with a considerable amount of basic research. Further studies in this area are needed to delineate these effects as to clinical significance.

2. Opiate alkaloid administration to depressed patients resulted in neuroendocrine findings of considerable importance to the understanding of the mode of action of opiates. In particular, a significant decrease in cortisol produced by methadone administration may be related to abnormalities in cortisol regulation found in depressed subjects. The one patient trial of oral methadone treatment was suggestive of a possible therapeutic effect of opiate agonist administration in depression.

3. The lack of significant behavioral effects produced by parenterally administered beta-endorphin may be attributed to the lack of drug access to the CNS. The findings of peripheral biological activities on the basis of neuroendocrine and radioreceptor assay findings are important methodologic contributions to this area of research.

4. The finding of a diurnal rhythm in CSF opioid activity in primates is consistent with other data regarding ACTH and other related peptides. This is the first such finding reported in the literature. We found no indication of elevations in CSF opioid activity among schizophrenic patients - this is in contrast to early speculation regarding increased endorphin activity in schizophrenia. Our findings relating CSF opioid activity to urinary free cortisol in depressed patients may contribute to further understanding of abnormalities in cortisol regulation in depression. The state related changes in opioid activity in patients with anorexia nervosa contributes to accumulating evidence suggesting a role for endogenous opioids in eating behavior.

Proposed Course:

1. A Phase II Naloxone Project coordinated by the Unit for the WHO in which neuroleptic treated patients will be administered naloxone for four consecutive days is currently underway. This may aid in evaluating the clinical significance of the initial findings. Furthermore, studies in the Unit will be addressing dose responsivity of naloxone pertaining to neuroendocrine parameters in order to establish an optimal naloxone dose for blockade of the endogenous opioid system.

2. Oral methadone treatment of refractory depressed patients will continue.

3. Because of the probable limited access of systemically administered beta-endorphin, the Unit is currently involved in assessing the possibility of administering beta-endorphin intrathecally to psychiatric patients. Evaluation of this technique will first be accomplished in a proposed administration of intrathecal beta-endorphin to intractable pain patients.

4. Continued analysis of data already generated in this project is currently in progress. This includes relating CSF opioid activity to

a variety of specific symptomatology within diagnostic groups. Further research in this area will attempt to delineate which of the various endogenous opioids may be deficited in schizophrenic patients.

Publications:

Naber, D., Pickar, D., Dionne, R., Bowie, D., Ewels, B., Moody, T., Soble, M. and Pert, C.: Assay of endogenous opiate receptor ligands in human CSF and plasma. Substance and Alcohol/Misuse 1: 83-89, 1980.

Schultz, S.C., Wagner, R., van Kammen, D.P., Rogol, A., Davis, G., Wyatt, R., Pickar, D., Bunney, W.E., Jr. and Li, C.H.: Prolactin response to beta-endorphin in man. Life Sciences 27: 1735-1741, 1980.

Gold, P., Extein, I., Pickar, D., Rebar, R., Ross, R. and Goodwin, F.: Acute intravenous methadone infusion suppresses plasma cortisol in depressed patients. American Journal of Psychiatry 137: 862-904, 1980.

Pickar, D., Cutler, N., Naber, D., Post, R., Pert, C. and Bunney, W.E., Jr.: Plasma opioid activity in manic-depressive illness. Lancet 1: 937, 1980.

Naber, D., Cohen, R., Pickar, D., Kalin, N., and Bunney, W.E., Jr.: Episodic secretion of opioid activity in human plasma and monkey CSF: Evidence for a diurnal rhythm. Life Sciences 28: 931-935, 1981.

Extein, I., Pickar, D., Gold, M.S., Gold, P.W., Pottash, A., Sweeney, D., Rebar,R., Martin, D. and Goodwin, F.: Methadone and morphine in depression. Psychopharmacology Bulletin 17: 29-33, 1981.

Pickar, D., Davis, G., Schulz, C., Extein, I., Wagner, R., Naber, D., Gold, P., van Kammen, D., Goodwin, F., Wyatt, R., Li, C.H. and Bunney, W.E., Jr.: Behavioral and biological effects of acute beta-endorphin injection in schizophrenic and depressed patients. American Journal of Psychiatry 138: 160-166, 1981.

Pickar, D., Naber, D., Post, R., van Kammen, D., Ballenger, J., Waters, R., Rubinow D., Goodwin, F. and Bunney, W.E., Jr.: CSF endorphins in psychiatric patients. Psychopharmacology Bulletin, in press.

Naber, D., Pickar, D., Davis, G., Cohen, R.., Jimerson, D., Elchisak, M. DeFraites, E., Kalin, N. and Buchsbaum, M.S.: Naloxone effects on beta-endorphin, cortisol, prolactin, growth hormone, HVA and MHPG in plasma of normal volunteers. Psychopharmacology, in press.

Cohen, M. and Pickar, D.: Pharmacological challenges to the endogenous opioid system in affective illness. Journal of Clinical Psychopharmacology, in press.

Post, R., Pickar, D., Naber, D., Ballenger, J., Uhde, T. and Bunney W.E., Jr.: Effect of carbamazepine on CSF opioid activity: Relationship to antidepressant response. Psychiatry Research, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00114-01 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Surgical Stress and the Endogenous Opioid System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Yolanda Roth, M.D.	Medical Oncology	NCI
William E. Bunney, Jr., M.D.	Chief	BP, NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH
Biological Psychiatry Branch

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Unit on Studies of Drug Abuse

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.25	PROFESSIONAL: 1.0	OTHER: 1.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project studies the effects of surgical stress on the endogenous opioid system. It is well known that surgical stress is associated with responses in cortisol as well as in catecholamines; changes in heart rate and blood pressure are also associated with surgical procedures. We have studied the effects of anesthesia induction, abdominal and limb surgery and post-operative morphine administration on plasma levels of beta-endorphin and opioid activity and their relationship to changes in cortisol and post-operative pain medication requirements.

PHS-6040
(Rev. 10-76)

Objectives

One of the variety of hypothesized roles of the endogenous opioid system in man is that of stress homeostasis. A number of biological systems which are responsive to surgical stress and post-operative behavioral abnormalities are well known. In this project, we have systematically studied the effect of surgery on the endogenous opioid system. A major goal of this project is to investigate the potential response of the opioid system to surgery and its relationship to post-operative pain.

Methods Employed

In a pilot study, six patients who underwent lower limb amputation for regional malignancy were studied with regard to changes in plasma beta-endorphin immunoreactivity and plasma opioid activity accompanying the surgical procedure. Blood samples were taken prior to, during and post surgery. In a second study, a series of 10 patients undergoing abdominal surgery for malignancy staging were studied. Blood samples were collected prior to anesthesia induction, post-induction, during surgery, when patients were awake in the recovery room, both prior to and after the administration of morphine.

Major Findings

In both studies, the surgery but not anesthesia induction were associated with large increases in plasma beta-endorphin immunoreactivity. In individual subjects in whom determinations were made, surgery but not anesthesia induction also was associated with robust increases in plasma opioid activity. Elevations in plasma beta-endorphin immunoreactivity were found post-operatively when the patients were awake; post-operative morphine administration produced significant decreases in plasma beta-endorphin immunoreactivity. Changes in beta-endorphin across all study times were highly correlated with changes in plasma cortisol. In eight subjects, in whom data was available, the total amount of post-operative morphine given on the basis of patient request was correlated with resting levels of plasma beta-endorphin immunoreactivity. In four subjects, in whom high dose fentanyl was given as an adjunct to halothane anesthesia, no increases in plasma beta-endorphin immunoreactivity or cortisol were observed to accompany surgery.

Significance to Biomedical Research

The results of this project have considerable importance to medical research. These data suggest that the endogenous opioid system is stimulated by surgical stress. Furthermore, we observed a correlation between plasma beta-endorphin in cortisol supporting previous animal data suggesting concomittant release of beta-endorphin and ACTH under stress. The finding of significant correlations between patient-requested post-operative morphine and resting plasma beta-endorphin immunoreactivity furthermore suggests that

levels of plasma beta-endorphin may be functional with regard to pain regulation and perception. The effect of high dose opiates as an adjunct to general anesthesia appears to be a suppression of a variety of stress responses including that of the endogenous opioid system.

Proposed Course

The Unit will continue to study the relationship of the endogenous opioid system to stress and to surgical pain. Future studies will include a relationship between catecholamine and endorphin response to surgical stress.

Publications

Cohen, M., Pickar, D., Dubois, M., Roth, Y., Naber, D., Bunney, W.E., Jr.: Surgical stress and endorphins. Lancet 1: 213, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00070-08 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Psychological and Biological Interactions in the Acute Psychoses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	R.M. Post, M.D.	Chief, Sect. on Psychobiology	BP NIMH
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	F.W. Putnam, M.D.	Staff Psychiatrist	BP NIMH
	W.H. Berrettini, M.D.	Clinical Associate	BP NIMH
	B. Scupi	Clinical Social Worker	BP NIMH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12.0	6.0	6.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients suffering from manic, depressive, schizoaffective, and anxiety disorders are longitudinally evaluated and treated. Double-blind placebo-controlled trials are conducted and have demonstrated that the anticonvulsant carbamazepine is useful in treating both manic and depressive illness. This discovery evolved from the use of animal models such as kindling and cocaine sensitization which provided clues to mechanisms underlying progressive development of behavioral pathology. Cocaine sensitization in animals is environmental context specific, related to conditioning variables, and modulated by neuropeptides and sex hormones. In patients with affective illness and normal volunteers, neurotransmitters and metabolites, hormones, and peptides implicated in the regulation of mood and behavior are measured in blood, urine, and cerebrospinal fluid as an approach to studying normal and pathological brain function. Alterations in cognitive function, neurophysiology, and biochemistry are explored in relation to clinical response to anticonvulsants, dopaminergic and noradrenergic receptor agonists, the paradoxical therapeutic effects of sleep deprivation in depression, and related psychobiological approaches to understanding and treating mood and anxiety disorders.

I. Project Description

A. Objectives

This project is engaged in the multidisciplinary longitudinal study and treatment of patients with a spectrum of acute and chronic psychoses, particularly involving mood disorders. Both investigative and treatment approaches focus on the elucidation of psychological and biological phenomena and their complex interaction.

B. Methods Employed

1. Subjects

a. Subjects who meet Research Diagnostic Criteria (RDC) for manic-depressive or schizoaffective illness or the more recent DSM III criteria for a spectrum of mood disorders are admitted to the 3-West Clinical Research Unit, Section on Psychobiology. Patients with anxiety, panic anxiety, and phobic disorders, as well as those with multiple personality syndrome, are also admitted to the unit under other protocols (see Project #Z01 MH 00071-01 BP and #Z01 MH 00072-01 BP). Depressed patients are rated for degree of typicality of depression on a formal rating scale developed in collaboration with Dr. E.K. Silberman.

b. Normal volunteers are also admitted to the unit to provide control data for specific studies in patients and to assess clinical and biological interrelationships in normal as well as patient populations. More than 42 volunteers have completed an extensive battery of biochemical, psychological, and physiological tests including lumbar punctures.

2. Psychological and Biological Evaluation

a. Behavior and Cognition. During an initial drug-free interval patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation, including EEG-monitored sleep, averaged evoked potentials, and a variety of cognitive tests. These include the Halstead Category Test, the Levine Test, the cognitive style questionnaire, a psychosensory questionnaire, and a neuropsychological profile designed to assess disturbances in brain function with the Luria Battery. Studies of specific disturbances in memory and learning are conducted with Drs. D.R. Rubinow, J.P. Boulenger, H. Weingartner, and E.K. Silberman.

Longitudinal behavioral data are collected in a double-blind fashion utilizing twice-daily global ratings by trained nursing observers. Patients also complete twice-daily ratings of mood and side effects in order to examine diurnal variation. Using the same double-blind methodology, nurses also evaluate patients on a modified Brief Psychiatric Rating Scale (BPRS) three times weekly.

b. Life Chart Methodology. A life chart technique has been developed to plot the number and severity and interval between affective episodes so that the longitudinal development, recurrence, and progression of the illness can be accurately quantitated and illustrated. This technique is an

important clinical as well as research tool for assessing the efficacy of treatment interventions.

c. Physiology. Motor activity is measured continuously at 15-minute intervals with a miniaturized activity monitor developed by Dr. T. Colburn. A modification of this monitor is being utilized by Dr F.W. Putnam to assess motor activity during sleep stages based on activity data recorded every 3.25 minutes. EEG-monitored sleep is studied in collaboration with Dr. J.C. Gillin and W. Duncan. In collaboration with Dr. M.S. Buchsbaum, 16 channel EEGs, average evoked responses, and studies of hemispheral laterality and psychophysiological pain are conducted.

d. Functional Anatomy. In addition to computerized axial tomography (CAT) scan evaluation of our patients for possible cerebral pathology, studies have been initiated in collaboration with Dr. M.S. Buchsbaum and a large number of associates to study regional functional activity of the brain using (18F) flurodeoxyglucose.

e. An Alpha-Adrenergic Agonist, Clonidine. Clonidine is administered intravenously to depressed patients and volunteers in order to assess clinical, physiological, and neuroendocrine responses to this alpha-adrenergic agonist (with Dr T.W. Uhde in collaboration with Drs. L. Siever and D.L. Murphy).

f. Alpha-Adrenergic Receptors. In collaboration with Dr. M. Kafka, platelet alpha receptor function, as well as prostaglandin-stimulated increases in cyclic AMP, are assessed in normal volunteers and patients with mood and anxiety disorders.

g. Urinary MHPG and Urinary Free Cortisol. These substances are evaluated in basal 24-hour urinary collections during depressed and manic states in medication-free conditions and during treatment.

h. Cerebrospinal Fluid (CSF) and Plasma Studies. Plasma and CSF studies compose a core area of biological evaluation of classical neurotransmitters and their amines as well as the newly discovered peptide substances in normal volunteers and in patients during ill and well intervals. These studies are conducted in collaboration with Drs. F.K. Goodwin, P.W. Gold, D.C. Jimerson, and M.H. Ebert, as well as many investigators outside of NIMH with specialized techniques for measurement of specific peptide hormones.

i. Oxytocin and Vasopressin. In collaboration with Drs. H. Weingartner, P.W. Gold, and Dr. D.R. Rubinow, infusions of these peptides are utilized to assess effects on memory, mood, and endocrine function in affectively ill patients and normal volunteers.

j. Dexamethasone Suppression. A detailed evaluation of the pituitary adrenal axis is conducted by Dr. D.R. Rubinow in patients with affective illness and anxiety disorders. Plasma and urinary free cortisol are measured under basal conditions and following the dexamethasone suppression test. Cortisol receptor binding on lymphocytes is assessed in collaboration with Dr. D.C. Jimerson. ACTH is measured in collaboration with Dr. D. Pickar.

3. Treatment

a. Psychotherapeutic. Treatment and evaluation are conducted in individual and group therapy, and ongoing clinical case conferences are utilized.

b. Routine Somatic Treatment. Both routine and experimental compounds are evaluated during double-blind clinical trials. The routinely used drugs include tricyclic antidepressants, lithium carbonate, monoamine oxidase inhibitors, and neuroleptics. These agents are utilized not only because of their clinical efficacy, but as well to further understand their mechanisms of action and possible interaction with the pathophysiology of the illness.

c. Experimental Compounds. The anticonvulsant carbamazepine has been introduced as a new treatment for manic and depressive illness and is evaluated for its acute and prophylactic efficacy. Diphenylhydantoin and valproic acid are two other anticonvulsant agents also being studied in selected patients to assess the specificity of the positive psychotropic effects of carbamazepine in relation to other anticonvulsants with different spectrums of clinical efficacy.

d. Receptor Agonists and Antagonists. The dopamine receptor agonist piribedil and the dopamine antagonist pimozide are utilized to study therapeutic response in relation to alterations in dopaminergic systems. Clonidine, in addition to acute intravenous studies, is administered during clinical trials in order to assess the clinical efficacy of alterations in adrenergic functioning in anxiety and affective illness.

e. Peptide Strategies. In addition to acute challenges with oxytocin and vasopressin, clinical trials have been conducted in collaboration with Dr. P.W. Gold of a vasopressin analog DDAVP in affective illness.

f. Sleep Deprivation. The paradoxical antidepressant effects of one night's sleep deprivation in depressed patients are explored both to develop a model for further understanding the rapid onset of a non-pharmacologically-induced mood improvement and to assess their therapeutic potential.

g. Animal Models. A rodent and primate behavioral pharmacology laboratory is maintained to develop new research techniques in several areas. The longitudinal evolution of behavioral pathology is assessed using a number of paradigms including: 1) electrophysiological kindling; 2) pharmacological kindling; 3) behavioral sensitization to psychomotor stimulants and related dopaminergic agonist compounds; and 4) the evaluation of stress sensitization and its possible underlying neural substrates. Physiological and biochemical changes, in particular alterations in receptor binding, are studied in collaboration with Drs. M. Del Zompo, J. Tallman, A. Pert, and C. Pert. ^{14}C-2-deoxyglucose studies have been conducted utilizing pharmacological kindling with lidocaine in collaboration with Drs. C. Kennedy, L. Sokoloff, and associates. The role of seizures in the development of psychopathology is studied utilizing a variety of seizure models, behavioral assessment, and anticonvulsant compounds.

C. Major Findings

1. Carbamazepine as a New Treatment for Manic-Depressive Illness

a. Rationale. Following the introduction of the use of carbamazepine for manic and depressive illness in the first double-blind clinical controlled trials, we have continued to document the efficacy of carbamazepine in affective illness. The rationale for the initiation of clinical trials is based on several theoretical and empirical factors. 1) There were consistent reports of mood improvement in epileptic patients treated with carbamazepine. 2) Carbamazepine has been shown to be particularly effective in epilepsy of the temporal lobe and limbic systems both clinically and in animal models; it is these substrates that have been linked to disorders of affect. 3) Carbamazepine inhibits kindled limbic seizures which have been utilized as a model for the progressive evolution of psychopathology. 4) Preliminary data in non-controlled studies in Japan indicated positive effects in patients with manic-depressive illness when carbamazepine was added to previously ineffective drug regimens.

b. Clinical Efficacy. Carbamazepine possesses clinical efficacy in both manic and depressed phases of the illness. Most notably, it appears to be effective in some patients who were previously non-responsive to lithium carbonate, the major tool for the acute and prophylactic treatment of affective illness. Manic patients in many instances have shown evidence of unequivocal response as demonstrated by repeated off-on-off-on experimental designs, i.e., improvement is demonstrated following institution of active treatment with carbamazepine, relapse may occur following placebo substitution, and the patient improves again when active treatment of carbamazepine is reinitiated.

Treatment with carbamazepine has also been associated with clinical improvement in the depressed phase of both bipolar and unipolar affective illness. In some instances improvement in depression has continued following drug withdrawal, while in the minority of cases depressive symptomatology recurs following double-blind drug discontinuation with placebo substitution. Sleep is significantly improved even during the first week of treatment with carbamazepine, as assessed by half-hourly nurse sleep checks. Formal EEG-monitored sleep studies in collaboration with Dr. J.C. Gillin indicate that the improvement in sleep is occurring in predominantly slow wave sleep (stages 3 and 4).

This improvement in sleep occurs without notable daytime sedation and the drug is well tolerated by affectively ill patients, consistent with the decreased incidence of side effects relative to other anticonvulsants. Although there are several reports in the literature of the development of aplastic anemia during treatment with carbamazepine, there has been a decline in these reports even though use of the drug for epilepsy has increased. Moreover, we have observed only mild white count suppression in our patients studied to date.

c. Prophylactic Efficacy. Our preliminary clinical experience suggests that carbamazepine may also have useful prophylactic effects against the recurrence of both manic and depressive episodes. Nine patients have been maintained on carbamazepine for periods ranging from 4 months to 2½ years. Nine out of ten of these patients had been previously non-responsive to lithium carbonate. The degree of clinical response to carbamazepine as the sole treat-

ment or given in combination with lithium or neuroleptics was good to partial to excellent in these patients.

A major component of the clinical trial is the clinical and/or biological identification of patients who may respond to carbamazepine in contrast with lithium carbonate or other routinely used agents. To date, responders to carbamazepine are not clinically distinguishable from others with affective illness; in particular, there is no evidence of a seizure disorder in these patients.

d. Blood and CSF Carbamazepine Levels. Carbamazepine is utilized in a dose range of 600-2000 mg/day, roughly the same clinical range used in the treatment of epilepsy. Blood levels have been maintained usually within a range of 8-12 µg/ml. Both blood and CSF levels of carbamazepine have not been related to the degree of clinical response. However, measurement of the carbamazepine metabolite, carbamazepine-10,11-epoxide, in collaboration with Drs. T.W. Uhde, D.C. Chatterji, and R.F. Green has revealed a positive correlation with the degree of clinical antidepressant or antimanic response. These studies thus suggest that the -10,11-epoxide, which has been reported to have active anticonvulsant properties in animal studies, may also have active psychotropic properties for the treatment of manic-depressive patients.

Carbamazepine thus forms the core element of a strategy utilizing anticonvulsant compounds including diphenylhydantoin and valproic acid to potentially develop a series of new treatments for affective illness, as well as to study the possible interrelationships between seizures and psychosis. A unique derivative of this work has been the conceptualization of electroconvulsive therapy (one of the most effective treatments for affective illness) as acting not by its seizure-inducing properties, but as possibly acting through its own anticonvulsant effects. As discussed below, electroconvulsive shock appears, like carbamazepine and valproic acid, to have major effects on inhibiting the development of amygdala kindling.

e. Carbamazepine's Effects on Classical Neurotransmitter and Peptide Systems

1) Classical Neurotransmitters. Biochemical studies are conducted in association with the ongoing clinical trials to elucidate how carbamazepine may be affecting neurotransmitter metabolites, as well as to determine possible biochemical correlates of clinical response. Preliminary data support earlier animal work that carbamazepine may affect noradrenergic metabolism since the norepinephrine metabolite MHPG is significantly reduced in spinal fluid of patients treated with the drug. The dopamine metabolite HVA is not significantly affected under basal conditions but probenecid-induced accumulations are reduced, providing further indication that carbamazepine is not acting as a traditional neuroleptic. Preliminary data in collaboration with Drs. J.C. Ballenger and P.W. Gold indicate that arginine-induced and TRH-induced increases in prolactin are significantly increased on carbamazepine compared to placebo. CSF 5-hydroxyindoleacetic acid (5HIAA) is not significantly decreased on carbamazepine treatment as it is with routinely utilized antidepressant agents. Alterations in gamma-aminobutyric acid (GABA) have been postulated to be involved in seizures, as well as in alterations in affective illness. Carba-

mazepine does not significantly affect GABA levels in CSF although this does not rule out a more discrete effect on GABA mechanisms.

Psychotropic and anticonvulsant drugs have also been postulated to act through their effects on cyclic nucleotides. Carbamazepine does not significantly affect basal levels of either cyclic AMP or cyclic GMP in the CSF of affectively ill patients, although probenecid-induced accumulations of these compounds are significantly reduced. Moreover, those patients with higher pretreatment basal levels of CSF cyclic GMP showed the most robust antidepressant effects to carbamazepine.

2) Opiates. In addition to carbamazepine's anticonvulsant effects and positive psychotropic effects in affective illness, it has been used in the treatment of acute and chronic pain syndromes including trigeminal neuralgia. Because of these findings and carbamazepine's ability to potentiate opioid-induced hyperactivity in mice, we were particularly interested in assessing the interaction of carbamazepine with possible endogenous opioid systems. CSF opioid binding activity was measured in collaboration with Drs. D. Naber, D. Pickar, and associates in 17 patients on and off carbamazepine. There was no significant effect of the drug on this global measure of opioid systems, although more discrete effects on individual opioid peptides cannot be ruled out at this time. Baseline pretreatment values of opioid binding activity in CSF were positively correlated ($r = .79$) with degree of clinical antidepressant response in the 13 depressed patients studied.

3) Somatostatin. Carbamazepine significantly decreased CSF somatostatin measured in collaboration with Drs. D.R. Rubinow, P.W. Gold, and S. Reichlin. The relationship of this decrease in somatostatin following treatment with carbamazepine to degree of clinical efficacy and/or side effects is currently being assessed. However, this report represents one of the first findings in man of a significant effect of psychotropic agents on the newly-discovered putative central nervous system (CNS) peptide neurotransmitters.

4) Cortisol. Preliminary studies in collaboration with Dr. D.R. Rubinow indicate that carbamazepine produces a disinhibition of the pituitary adrenal axis, is associated with increases in plasma cortisol and urinary free cortisol, and may induce escape from dexamethasone suppression in a small number of patients studied to date. These data may be of importance in understanding the relationship between affective symptomatology and pituitary adrenal axis disinhibition frequently seen in depressed patients. Carbamazepine thus represents one of the first psychotropic agents to dissociate improvement in mood from normalization of cortisol hypersecretion. As such, the cortisol findings may be important in understanding the mechanism of action of carbamazepine, as well as useful in considering the relationship of hypercortisolemia to depression and its possible pathophysiology.

5) Vasopressin. Carbamazepine, in contrast to lithium carbonate which has inhibitory effects on vasopressin activity and may produce diabetes insipidus, appears to have vasopressin agonist effects in man. Drs. P.W. Gold and J.C. Ballenger have noted altered vasopressin response to a hypertonic saline load during carbamazepine treatment; findings in the opposite direction were observed during treatment with lithium carbonate. Consistent

with a vasopressin agonist effect either directly or indirectly, Dr. T.W. Uhde has noticed statistically significant hyponatremia during carbamazepine administration and rapid reversals of this effect following carbamazepine withdrawal. The relationship of carbamazepine's effects on vasopressin to its mood and behavioral response in affective illness remains to be explored. However, the drug may prove to be of use particularly in patients who have difficulties with lithium-induced diabetes insipidus, as carbamazepine has previously been clinically used for the treatment of this syndrome.

2. GABA in Affective Illness and Anxiety

Studies conducted in collaboration with Dr. W.H. Berrettini have been initiated to further assess the possible role of alterations in GABA metabolism with mood and affective disturbances. A variety of indirect pharmacological data support a possible role of GABA in affective illness. Manipulations of the GABA system have also been shown to reverse depression-like behavior in models such as learned helplessness. Values of GABA in CSF have not been significantly decreased in depressed patients as a group compared to normal volunteer controls, although values in individual patients studied longitudinally are significantly lower in depressive compared to manic phases of the illness. Dr. W.H. Berrettini has developed an assay for GABA transaminase (GABA-T) in platelets and has examined the role of plasma GABA in affectively ill and normal patients. Preliminary evidence suggests that GABA-T is a relatively stable enzyme in normal volunteers studied longitudinally and is significantly correlated in twin pairs studied in collaboration with Drs. J.I. Nurnberger and E.S. Gershon. Possible state-related fluctuations in plasma GABA or in platelet GABA-T are currently being evaluated in rapidly cycling patients. Preliminary data suggest that plasma and CSF GABA levels are not significantly correlated, suggesting possible differential synthesis, metabolism, and/or transport of GABA in these two body fluids. Dr. W.H. Berrettini, with Drs. N. Rosenthal and F.K. Goodwin, has observed 50% elevation in plasma GABA 15 minutes after active but not placebo electroconvulsive therapy. Clinical studies in affectively ill patients of valproic acid (an anticonvulsant thought to act by increasing functional GABA activity in brain) have been initiated.

3. Norepinephrine and Its Metabolites in Affective Illness

a. In manic-depressive patients studied longitudinally urinary MHPG, plasma MHPG, and CSF MHPG are significantly higher in individual patients during their manic phase compared to the depressed phases of the illness. These findings are observed even though group comparison data with normal volunteers do not always reveal significant differences. In addition, CSF norepinephrine, measured directly by sensitive radioenzymatic assay in collaboration with Dr. C.R. Lake, is significantly elevated in manic patients compared to depressed patients and normal or neurological controls. CSF norepinephrine may actually be significantly elevated in depressed patients compared to normal volunteers, contrary to the catecholamine hypothesis. It is of interest, however, that depressed patients with higher ratings of anxiety had higher levels of CSF norepinephrine. These data are consistent with those of Drs. J.C. Ballenger, D.C. Jimerson, and associates indicating that in normal volunteers CSF MHPG is significantly correlated with degree of anxiety prior to the lumbar puncture.

b. Plasma MHPG in Depression and Normal Volunteers. Plasma MHPG, measured in collaboration with Dr. D.C. Jimerson, in 21 normal volunteers was significantly and negatively correlated with the severity of depression, hypochondriasis, and psychasthenia profiles measured by the Minnesota Multiphasic Personality Inventory (MMPI). These significant inverse correlations ($r = -.45$ to $-.50$, $p < .05$) with plasma MHPG represent the first report of an association of normal variations in depressive and associated affects with noradrenergic function. These data raise the possibility that there may be an association between norepinephrine and depressive affect in the normal as well as in the pathological range as previously observed in our longitudinal studies of patients. The precise nature of this interrelationship of plasma MHPG and depressive mood in volunteers will be important to clarify, particularly since MHPG is significantly correlated within twin pairs studied in collaboration with Drs. J.I. Nurnberger and E.S. Gerhson and thus could reflect a "trait" phenomenon.

c. Interrelationships of Urinary, Plasma, and CSF MHPG in Normals. These studies, in collaboration with Drs. D.C. Jimerson, J.C. Ballenger, C.R. Lake, F.K. Goodwin, and I.J. Kopin, have indicated important interrelationships between different measures of noradrenergic function. CSF MHPG was significantly correlated with plasma free and plasma conjugated MHPG. Moreover, CSF norepinephrine and plasma free and plasma conjugated MHPG were also significantly correlated. However, CSF and plasma measures were poorly correlated with urinary measures of MHPG. These data further support the utility of plasma MHPG as a possible marker of noradrenergic activity and tend to add to the increasing evidence that urinary MHPG may not necessarily be a useful marker of central noradrenergic function.

d. Alpha-2 Receptor Agonist, Clonidine. Endocrine and behavioral responses to an acute intravenous challenge of clonidine have been explored in normal and depressed patients in collaboration with Drs. T.W. Uhde, L.J. Siever, and D.L. Murphy. Significant decreases in plasma norepinephrine and MHPG have been observed in association with a significant decrease in blood pressure. Growth hormone responses appear to be blunted in a substantial number of depressed patients, particularly post-menopausal females, although the blunted responses are also observed in the normal control population. The utility of this endocrine marker for depressed patients is being explored in a larger patient and volunteer sample size.

Substantial antianxiety effects were observed by Dr. T.W. Uhde in depressed patients treated with clonidine. These antianxiety effects were not evident during the placebo infusion and support a variety of other data regarding the possible interrelationships of anxiety and noradrenergic function and the possible treatment of anxiety with clonidine. The interactions of clonidine, measures of MHPG, anxiety, beta-endorphin, and psychophysiological pain are discussed by Dr. T.W. Uhde in Project #Z01 MH 00071-01 BP.

4. Dopaminergic Mechanisms in Mania and Depression

a. Both indirect pharmacological data and assessment of the dopamine metabolite HVA in affectively ill patients support a possible role for alterations in dopaminergic systems in affective illness. The dopamine agonist

piribedil in moderate doses has been observed to have antidepressant effects, while pimozide and related neuroleptics which block dopamine receptors are effective in treating mania. Recent data from other laboratories also suggest that a variety of antidepressant treatments may have a common effect in desensitizing presynaptic inhibitory receptors as measured by biochemical endpoints (Gessa and associates) or studied by electrophysiological recording of dopamine neurons (Antelman and associates). These findings are also consistent with our evidence that HVA may be decreased in a subgroup of depressed patients and that manic patients may show greater neuroleptic-induced HVA accumulations following probenecid treatment than non-manic patients.

b. Studies in collaboration with Drs. N.R. Cutler and W.E. Bunney, Jr. in 17 subjects have demonstrated diurnal variation in dopaminergic responsivity utilizing the hypothermic response to apomorphine at 9:00 a.m. compared to 9:00 p.m. Greater hypothermic effects were observed in the evening compared to morning although differences in affective, sedative, and endocrine effects were not significantly different at these two time periods. The temperature response to apomorphine may provide an index of hypothalamic dopaminergic responsivity in man. Following repeated administration of apomorphine, a significant increase in the rebound hyperthermia is observed compared to the initial apomorphine response. This increased responsivity to a dopaminergic agonist challenge in man may represent an important adaptation to repeated activation of the dopaminergic system.

c. Tardive Dyskinesia in Affective Illness. In collaboration with Drs. N.R. Cutler, A.C. Rey, and W.E. Bunney, Jr., we have observed marked alterations in the degree of tardive dyskinesia associated with mood phase in rapidly cycling manic-depressive patients. Tardive dyskinesia was markedly worse in depressive phases of the illness, improved with the onset of mania, and was exacerbated with the onset of the next depressive episode in patients studied repeatedly over a number of manic-depressive cycles. These findings suggest then an association between the biological processes underlying depressive illness and exacerbation of tardive dyskinesia. The findings are also contrary to those based on current theories of tardive dyskinesia and affective illness that would predict an exacerbation of tardive dyskinesia during mania rather than depression if the postulated dopaminergic supersensitivity of tardive dyskinesia were interacting with the increased dopaminergic function thought to be associated with mania.

5. Sleep Deprivation in Depressed Patients and Normal Volunteers

Since our initial studies initiated in collaboration with Drs. J. Kotin and F.K. Goodwin in 1973, we have continued to document the paradoxical and substantial acute antidepressant effects of deprivation of one night's sleep in depressed patients in contrast to the usual mild increases in dysphoria in normal volunteers. These data continue to be clinically and theoretically provocative, suggesting a differential mood response in depressed patients compared to volunteers which ultimately might lead to the elucidation of basic differences in underlying biological substrates. We are currently evaluating the clinical efficacy of repeated sleep deprivations in patients in a medication-free state and in combination with pharmacological treatments. Markers of a positive sleep deprivation response include initial greater severity of de-

pression, initial greater severity of depressive sleep disturbance (in collaboration with W. Duncan and Dr. J.C. Gillin), greater typicality of the depressive presentation (studied in collaboration with Dr. E.K. Silberman), and alterations in several of evoked potential measures (studied with Dr. M.S. Buchsbaum).

The degree of clinical antidepressant response to one night's sleep deprivation is significantly correlated with the degree of relapse following one night's recovery sleep. The return of depressive affect and behavior following a night's sleep provides a unique opportunity to investigate the physiological, hormonal, and biochemical concomitants of this sleep-induced emergence of depressive symptomatology. Initial studies in collaboration with Drs. T.W. Uhde, F.W. Putnam, D.R. Rubinow, J.C. Gillin, and W.E. Bunney, Jr. indicate that as brief a period as 1.5 minutes of EEG-monitored sleep is sufficient to transiently reverse the sleep deprivation-induced improvement in depressive affect. The biological and physiological concomitants of this process are currently being investigated.

Endocrine studies conducted in collaboration with Dr. E. Weitzman utilizing 20-minute plasma sampling techniques in patients before, during, and after sleep deprivation have suggested that abnormal cortisol and growth hormone patterns may not be normalized following sleep deprivation even when it is associated with a complete lifting of depression.

6. The Relationship of Putative CNS Neurotransmitter Peptide Substances to Classical Neurotransmitters and Behavior

Rapid advances are occurring in the study of the relationships between CNS peptides and classical neurotransmitters. In some instances neuropeptides and classical neurotransmitters appear to be co-stored and co-secreted in the same neurons (Hokfelt, 1980). Both types of substances are being studied in plasma and CSF of patients and volunteers to further elucidate possible relationships of these compounds to each other as well as to possible aberrations in behavior.

a. ACTH (Cortisol) and Opioid Peptides. We continue to document evidence of pituitary adrenal axis disinhibition (ACTH hypersecretion) as evidenced by increases in plasma urinary free cortisol in depressed phases of affective illness compared to manic phases or to normal volunteers (studied in collaboration with Drs. D.R. Rubinow, P.W. Gold, and J.C. Ballenger). These data are consistent with a growing body of evidence that escape from dexamethasone suppression may occur in a substantial percentage of depressed patients. However, in some patients demonstrating evidence of cortisol hypersecretion based on 20-minute catheter samples and total excretion of urinary free cortisol, we have observed normal suppression following dexamethasone suppression. These data and the fact that approximately 50% of depressed patients in the literature do show normal responses to dexamethasone raise interesting questions regarding the nature and anatomical location of the regulatory defect in at least a subgroup of depressed patients. It is noteworthy that relationships between cortisol and affect were noted in normal volunteers studied by Dr. J.C. Ballenger. Low plasma cortisol was associated with increased scores on the mania scale of the MMPI, while higher levels of urinary free cortisol were associated with higher depression scale scores.

Total CSF opioid binding activity and immunoreactive beta-endorphin were measured in the CSF of patients and normal controls in collaboration with Drs. D. Pickar, D. Naber, F.K. Goodwin, and W.E. Bunney, Jr. No significant differences among affectively ill populations and normal controls were observed in either measure. However, in depressed patients CSF opioid binding activity was significantly and positively correlated with the degree of rated anxiety. Opioid binding activity and a measure of acute anxiety at the time of the lumbar puncture in normal volunteers were inversely correlated. This differential correlation of anxiety and opioid activity in different patient populations suggests possible interrelationships between endogenous opioid systems and different measures of anxiety in these subject populations.

Moreover, a differential relationship between opioid binding activity and urinary free cortisol was observed in depressed patients and normal volunteers. In the depressed patient population (particularly among those who showed evidence of high levels of urinary free cortisol), CSF opioid binding activity was significantly and positively correlated with urinary free cortisol, while no significant relationship was observed in normal volunteers. These data suggest that increased activation of the pituitary adrenal axis, as evidenced by cortisol hypersecretion, may be associated with concomitant elevations in CSF opioid binding activity, supporting a variety of clinical and animal data suggesting co-secretion of ACTH and opioid substances.

b. CSF Somatostatin. CSF somatostatin has been measured in affectively ill patients and normal volunteers by sensitive radioimmunoassay in collaboration with Drs. S. Reichlin, P.W. Gold, and D.R. Rubinow. Dr. D.R. Rubinow has found non-significant decreases in CSF somatostatin in depressed patients compared to those in the manic phase of the illness or normal volunteers. In individual patients studied longitudinally, somatostatin levels were significantly lower in depressed compared to manic phases of the illness. These data also parallel the observation that normal volunteers with a history of minor depressions or episodic minor depressions had significantly lower CSF somatostatin levels than those without such a prior history. CSF somatostatin was not significantly correlated with the severity of depression, anxiety, or psychosis in this patient population but, consistent with the animal data, was significantly and inversely associated with degree of sleep disturbance. Affectively ill patients with the greatest degree of sleep disturbance had higher levels of CSF somatostatin. These data suggest that somatostatin alterations may be associated with disturbances in different symptom areas if not with depressive illness per se. Consistent with the postulated relationships between somatostatin and classical neurotransmitter systems, significant positive correlations between somatostatin and CSF MHPG and 5HIAA have been observed. These latter findings are of particular interest in relation to the reports of coexistence and co-secretion of CSF somatostatin and norepinephrine. As noted above, carbamazepine significantly decreased CSF somatostatin independently of its degree of therapeutic efficacy.

c. Vasopressin. In collaboration with Drs. P.W. Gold, J.C. Ballenger, H. Weingartner, D.R. Rubinow, and G. Robertson, vasopressin levels in CSF and its effects on mood and cognition have been explored. Preliminary data suggest that a vasopressin analog 1-desamino-8-D-arginine vasopressin (DDAVP) is associated with selective areas of memory improvement in affectively ill pa-

tients and normal volunteers. Although two psychotically depressed patients showed marked elevations in CSF vasopressin, values in non-psychotic bipolar depressed patients tended to be lower than those in a manic phase or in normal volunteers. The comparative effect of vasopressin and oxytocin on mood and memory is currently being explored.

d. Other Peptides in CSF. We have recently reviewed the evidence that almost all of the peptides that have been postulated to have a CNS origin are measurable in CSF. In addition to those peptides that were noted above, we have measured CSF calcitonin (in collaboration with Drs. K. Becker and J.S. Carman), angiotensin (in collaboration with Drs. P.W. Gold and D. Ganten), oxytocin (in collaboration with Drs. P.W. Gold and D. Fisher), and arginine vasotocin (in collaboration with Drs. D.R. Rubinow, P.W. Gold, and D. Fisher). In this fashion, it is hoped that systematic information regarding the interrelationships of neuropeptides, classical neurotransmitters, and normal and pathological behavior can be carried out whether or not any of these newly-discovered peptide substances is significantly altered in a given neuropsychiatric syndrome. We have reviewed the indirect evidence in the literature that suggests that peptides circulating in the CSF may have active neuroregulatory and behavioral effects. Even if the CSF does not prove to be an active neuroregulatory pathway and spinal fluid neuropeptides prove only to be an indirect marker of peptide function, it would appear that assessment of their possible biological and functional relationships in man will advance our understanding of these important neurotransmitter-modulator candidates.

7. Clinical Phenomenology

a. Life Course of Illness. We have instituted extensive longitudinal studies to characterize discretely defined affective episodes by standard criteria that occurred before, during, and after NIH hospitalization. A team approach is utilized in collecting these data with collaboration between patients, physicians, family members, social workers, and nurses. All other resources including past hospital records are used. In this fashion a systematic profile is established by final consensus with the patient of the longitudinal course of affective episodes. This data base, as established in more than 60 patients, will allow three phases of study. 1) The clinical phenomenology and longitudinal course of affective illness can be studied in relation to a variety of clinical and demographic factors. 2) Treatment evaluation can be based on more accurate assumptions about the pretreatment course of illness. 3) Relationships of course of illness to underlying biological mechanisms can be systematically explored.

Preliminary analysis of the data in the first phase has revealed marked differences in course of illness in bipolar compared to unipolar patients. A striking sex finding has also been observed with women being disproportionately higher represented in the group of rapidly cycling patients. Bipolar females had significantly greater numbers of affective episodes in the year prior to NIH hospitalization compared to men. These data raise interesting questions for further study regarding the increased vulnerability to extremely rapidly cycling manic-depressive illness in women compared to men and the possible endocrine concomitants of this vulnerability. Our life chart data furthermore substantiate the multiple studies since the time of Kraepelin which have emphasized

that recurrent affective illness tends to run a course of increasing rapidity of cycling in the majority of patients studied. This appears to be the case in a substantial number of patients even since the advent of lithium carbonate therapy. In addition, we have documented that patients with a greater number of previous episodes have more precipitous onset of individual episodes of mania, further suggesting a qualitative as well as quantitative modification in the course of illness following repeated episodes. Based on our models of cocaine-induced behavioral sensitization and electrophysiological kindling (discussed below), several possible mechanisms have been suggested to account for the apparent sensitization effects observed in recurrent affective illness. Specific hypotheses have been formulated and can now be tested. Moreover, it is noted that this longitudinal conception of affective illness and its possible parallels to sensitization and kindling models have already led to initiation of clinical trials of a new treatment for affective illness using the anticonvulsant carbamazepine.

b. Psychosensory Phenomena. Studies in collaboration with Dr. E.K. Silberman continue to document a high incidence of symptoms usually associated with temporal lobe epilepsy in patients with affective illness. The clinical and theoretical implications of these findings, as well as their possible relationship to response to the anticonvulsant carbamazepine, will be explored.

c. Atypicality of Depression. Systematic assessment of how "atypical" is the presentation of depression in patients who meet formal Research Diagnostic Criteria or DSM III criteria for affective illness has proved useful in the clinical, biological, and treatment-response prediction assessment of patients with affective illness. Unipolar and bipolar II patients show higher atypicality scores compared to bipolar I depressed patients. Older depressed patients are less atypical than younger patients. The more atypical patients had an increased number of total hospitalizations, although more typical patients showed rapid cycling in the year prior to NIH admission. Atypical depressed patients showed increasing measures of biological variance, particularly in the noradrenergic measures. Typical and atypical patients were also different on sleep parameters studied in collaboration with Dr. J.C. Gillin. Response to pharmacological treatment also differed between the two groups with typical patients also showing more positive response to sleep deprivation.

8. Multifactorial Approach to Affective and Cognitive Symptomatology

An example of the interdisciplinary approaches utilized within the Section on Psychobiology focuses on the assessment of cognitive disturbances in depressed patients. The Halstead Category Test provides one measure of abstracting ability in patients. The test uses designs and positional relationships and other aspects of cognitive processing that appear to tap right hemispheral function rather than the usual verbal measures of learning and information processing which appear to be relatively more dependent on left hemispheral function. Dr. R. Savard has observed striking impairment in cognitive function, as measured by the Halstead Category Test, in affectively ill patients compared to appropriate age- and socioeconomically-matched controls. In particular, bipolar depressed patients show more impairment than unipolar patients, and older bipolar patients showed marked and uniform increases in dysfunction of this mea-

sure. In addition, preliminary analyses conducted by Dr. D.R. Rubinow demonstrate that some of the variance is also accounted for by a variety of other clinical and biological measures. For example, increased levels of cognitive impairment are associated with patients who show: a) a greater number of prior affective episodes based on the life chart methodology; b) greater disinhibition of the pituitary-adrenal axis as measured by increased levels of excretion of urinary free cortisol; and c) evidence of abnormal cerebral pathology based on preliminary reading of the ventricular brain ratio based on CAT scan data. Systematic dissection of the interrelationships of these variables and their contribution to the overall cognitive dysfunction associated with depression should prove enlightening and useful from both a clinical and theoretical perspective.

A second example of this multifactorial approach derives from the first depressed patient studied with the positron emission tomography (PET) scan technique at the NIMH in collaboration with a large variety of investigators within and without the Institute including Drs. M.S. Buchsbaum, R.M. Kessler, W.E. Bunney, Jr., L. Sokoloff, T.W. Uhde, and D.R. Rubinow. The depressed patient showed increased metabolic activity as measured by glucose utilization in the left temporal parietal area. This finding in a single case was particularly provocative in light of independent confirmation by psychological testing utilizing the Luria Battery that the patient had disturbed cognitive functions usually associated with left temporal and parietal areas. Topographic, 16 channel EEG studies, in collaboration with Dr. M.S. Buchsbaum, demonstrated altered EEG patterns when the patient was studied during a severe depression compared to restudy in the medication-free improved state. It is noteworthy that the abnormality of her pituitary adrenal axis also normalized with her improved phase. Her markedly impaired performance on the Halstead Category Test likewise improved. These data in a single patient obviously have to be pursued in a large series of depressed patients, but the results in a single patient are interesting because of the correspondence of areas of dysfunction based on PET scan measures of glucose utilization, electrophysiology, psychological test profiles, and the associated dramatic alterations in clinical mood state. Pursuit of this multidimensional strategy should provide a powerful framework for integrating the results of further studies of the psychological, biochemical, and physiological concomitants of affective dysregulation.

9. Laboratory Models: Studies of the Progressive Development of Psychopathology and the Interaction of Seizures and Abnormal Behavior

a. Electrophysiology (In Collaboration with Dr. F.W. Putnam and N.R. Contel). Repeated, intermittent electrical stimulation of the brain results in increasing duration, spread, and complexity of electrical after discharges culminating in the appearance of a major motor seizure to a previously subthreshold stimulation. This altered responsivity leading to seizures, as discovered by Goddard and associates and named kindling, appears to be a relatively permanent alteration in brain excitability. We have observed a variety of associated behavioral and electrophysiological concomitants of this process. A subgroup of rats show regular rhythmic oscillations in amygdala excitability following kindling. This may represent a useful model for the induction of alterations in limbic excitability in experimental animals. Amygdala-kindled animals compared to sham-stimulated controls show less spontaneous and cocaine-

induced hyperactivity, but greater lidocaine-induced convulsive responsivity. These data suggest that long-lasting alterations in behavioral and convulsive responsivity occur following electrophysiological kindling.

b. Electroconvulsive Shock (ECS) Inhibits Amygdala Kindling. Amygdala-kindled animals were pretreated six hours prior to stimulation with either ECS or sham ECS. A third group of animals was treated with ECS immediately after the termination of the kindled after discharge. Marked inhibition of the development of amygdala-kindled seizures was observed in the animals given ECS six hours prior to amygdala kindling, compared to sham ECS animals or those receiving ECS immediately following termination of amygdala-kindled after discharge. These data suggest that ECS in animals may possess limbic anticonvulsant effects of equal magnitude to routinely used pharmacological anticonvulsants. These data also raise the possibility that the efficacy of electroconvulsive therapy in patients could be related to its limbic anticonvulsant properties. The mode of action of ECS in affective illness remains to be delineated, but the current studies raise a new and paradoxical theoretical conception regarding its possible mechanism of action -- that electroconvulsive seizures themselves may be associated with anticonvulsant effects.

c. Lidocaine-Induced Pharmacological Kindling

1) We have recently completed a series of studies documenting that the progressive development of seizures following lidocaine administration are not related to alterations in lidocaine pharmacokinetics and are probably mediated by a kindling-like phenomenon similar to that observed following electrophysiological kindling. Plasma lidocaine levels are not increased in animals displaying chronic lidocaine-induced seizures compared to a variety of other acute and non-treated control groups. Moreover, the blockade of lidocaine-induced seizures by co-treatment with diazepam prevents the development of the seizure sensitization effect. These data suggest that repeated activation of limbic structures with lidocaine is required in order to produce the sensitization to lidocaine-induced seizures.

2) Not only does electrical kindling cross-sensitize to lidocaine seizures, but animals experiencing lidocaine seizures show faster electrical kindling than non-seizing controls. These studies further suggest shared mechanisms of electrical and lidocaine-induced kindling.

3) In studies in collaboration with Drs. L. Sokoloff, C. Kennedy, their associates in the Laboratory of Neurochemistry, and K. Squillace, we have demonstrated that lidocaine seizures selectively activate limbic structures producing marked increases in glucose utilization in amygdala and hippocampus or in perirhinal and cingulate cortical areas.

4) Behavioral studies in collaboration with N.R. Contel and I. Pitem also indicate that long-lasting increases in aggression and irritability occur only in rats experiencing repeated lidocaine-induced seizures of the limbic system. Lidocaine-treated animals which do not seize and those co-treated with diazepam do not show the increases in irritability. Moreover, the increase in irritability appears to be related to lidocaine's selective activation of limbic areas, as this behavioral pattern is not noted following seizure-

induced ECS or metrazol which have a different pattern of effects and are not selectively related to limbic structures. This chemical model for the production of seizures of structures related to the limbic system may be useful in further systematic exploration of the relationship to altered behavior.

5) Studies in the rhesus primate of lidocaine- and procaine-induced behavioral effects and associated seizures are also in progress in collaboration with Drs. F.W. Putnam and W. Mendelson, utilizing depth electrode recordings from a variety of limbic structures in association with behavioral measures and concomitant CSF collection for analysis of neurotransmitter and peptide alterations. Preliminary results suggest that procaine-induced seizures in the rhesus primate are activating limbic structures and are associated with bizarre patterns of visual staring and searching behavior. A comparison with other local anesthetics including cocaine is in progress.

d. Cocaine-Induced Behavioral Sensitization. Behavioral sensitization to psychomotor stimulants and dopamine agonists provides another model for the increasing development of psychopathology. These studies are conducted with relatively low doses of psychomotor stimulants (cocaine 10 mg/kg, i.p.) in the rat and indicate that a variety of behaviors including locomotor hyperactivity and stereotypy show progressive augmentation to repeated administration of the same dose over time. This response is persistent, lasting more than two months following termination of chronic injections. Possible neurotransmitter and receptor alterations underlying this phenomenon are currently being investigated.

1) Conditioning and Environmental Context Dependency. We have replicated initial observations that cocaine-induced behavioral sensitization interacts with the environmental context in which the animal receives cocaine injections and is thus subject to conditioning variables. In particular, animals treated with cocaine in the context of the test cage apparatus show greater behavioral responsivity than animals treated with identical doses of cocaine in a different environmental context (their home cage). The importance of the role of conditioning factors in responsivity to psychomotor stimulants and related "stresses" is thus suggested. These findings may also be of heuristic value in considering the role of environmental stresses and conditioning variables in the progressive development of psychopathological conditions such as manic-depressive illness.

2) The Role of Vasopressin. Consistent with the observations that behavioral sensitization resembles other learning and memory paradigms affected by vasopressin, we have observed a differential rate of behavioral sensitization in animals deficient in vasopressin. Brattleboro homozygote animals which lack vasopressin showed deficient onset, maintenance, and persistence of cocaine-induced behavioral sensitization compared to their heterozygote litter-mate controls which do not have diabetes insipidus. These studies suggest that vasopressin deficiency or its downstream biochemical alterations may be associated with an altered rate of behavioral sensitization, consistent with a role for vasopressin in other models of learning and memory, including the development of drug-induced tolerance.

3) Endogenous Opiates in Behavioral Sensitization. Preliminary data suggests that endogenous opiate systems may be involved in the development of behavioral sensitization. Naloxone administration appears to inhibit the progressive development of cocaine-induced horizontal motor activity but facilitates vertical rearing activity. Further studies are indicated in order to determine which opiate systems and which discrete aspects of behavioral sensitization are affected.

4) Sex Hormones in Behavioral Sensitization. Female, compared to male, rats are acutely more responsive to the same dose of cocaine. It appeared as if female rats were already "pre-sensitized" and they thus did not appear to manifest cocaine-induced sensitization. However, when female animals were challenged with half the dose of cocaine (5 mg/kg, i.p.) they showed normal development of behavioral sensitization. These data suggest that female rats, possibly modulated through the effect of female sex hormones on dopamine and other neurotransmitter systems, are more sensitive to behavioral alterations induced by cocaine. These findings may be of interest in light of differences in males and females in the incidence and progression of the mood disorders, particularly given the disproportionate representation of female patients among those showing extremely rapidly cycling manic-depressive illness.

D. Proposed Course of Project

We will attempt to delineate clinical and biological markers of carbamazepine response in patients with manic-depressive illness, schizoaffective illness, and the anxiety disorders. A series of patients will be studied following determination of carbamazepine response in order to ascertain whether they also respond to other anticonvulsive agents including diphenylhydantoin and valproic acid. The use of anticonvulsant agents with a different clinical and biochemical spectrum of action should help differentiate the mechanisms by which carbamazepine may be exerting its clinical efficacy in affective illness. Assessment of biological changes in patients during treatment with carbamazepine will continue in order to assess relationship to clinical response. In particular, the fact that carbamazepine increases urinary free cortisol and induces escape from dexamethasone suppression will be studied, since pituitary-adrenal axis disinhibition appears to be one of the most robust markers of depressive illness. A variety of other effects of carbamazepine on vasopressin, somatostatin, and alpha-adrenergic receptor systems in platelets will also be pursued.

As part of the investigation of the possible locus of action of carbamazepine, studies of intravenous procaine hydrochloride will attempt to ascertain whether this local anesthetic which activates limbic structures is useful in predicting clinical anticonvulsant response. Profiles of neuropsychiatric dysfunction and regional neuroanatomical disturbance assessed by 16 channel EEG and PET scan techniques will also be explored in relation to life course of illness, degree of cognitive disturbance, and prediction of carbamazepine response. We will also assess whether our affectively ill patients who present with increased psychosensory phenomena that usually are associated with patients with temporal lobe epilepsy have a differential response to lithium carbonate or carbamazepine.

The preliminary findings that the metabolite carbamazepine-10,11-epoxide may possess active psychotropic properties in man as well as anticonvulsant properties in animals will be explored. The effects of carbamazepine on animal models of the progressive development of psychopathology, including kindling and behavioral sensitization, will be initiated. Further assessment of the mechanisms of the paradoxical anticonvulsant effects of electroconvulsive shock will be pursued.

The clinical markers of therapeutic effects in response to sleep deprivation as well as possible underlying mechanisms will continue to be studied. In particular, a variety of neurotransmitters, peptides, and neurohormones will be measured in plasma during sleep deprivation and sleep-induced alterations in mood and behavior.

Studies of the classical neurotransmitters as measured in plasma and CSF will continue in conjunction with determination of their relationship to the neuropeptides and how both classes of compounds may relate to normal and pathological behavior. Effects of selected peptides such as oxytocin and vasopressin on mood, memory, and behavior will also be pursued. Initial studies by Dr. W.H. Berrettini, indicating a vasopressin binding site on platelets, will be extended. Studies of the alpha-adrenergic agonist clonidine and its effects on depressed mood as well as on anxiety will be continued.

Under a separate protocol Dr. T.W. Uhde will more specifically assess neurotransmitter function in patients with phobic anxiety and panic anxiety syndromes. Response to treatment with clonidine and carbamazepine will be assessed in outpatient clinical trials. Studies of patients with multiple personality syndrome will be pursued by Dr. F.W. Putnam in relation to underlying psychological, psychophysiological, and neural mechanisms. Biological, physiological, and psychological studies of patients with menstrually-related exacerbation of mood and behavior will be initiated by Dr. D.R. Rubinow and conducted in close coordination with ongoing inpatient studies of patients with affective illness and anxiety disorders.

In each of the areas noted above it is hoped that the interdisciplinary approach to the assessment of psychological and biological change in patients with psychiatric disorders will allow the delineation of aspects of the underlying pathophysiology of these illnesses and also lead to the development of new and better treatment strategies. Studies in the animal laboratory will continue, particularly focused around the induction of long-term behavioral changes following repeated electrophysiological, chemical, and psychosocial stimulation. Exploration of the interactions of seizures and psychopathology and the mechanisms underlying a variety of sensitization and kindling phenomena have already led to the introduction of carbamazepine as a new treatment strategy for recurrent manic and depressive illness.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00071-01 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Psychobiological Correlates and Treatment of Pathological Anxiety

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with pathological degrees of anxiety and panic disorder meeting DSM III criteria are evaluated using psychological, physiological, and biochemical approaches to understanding and treating this disorder. Particular attention is given to the role of the noradrenergic transmitter system as assessed by: 1) measurement of the metabolite MHPG in urine, plasma, and CSF; 2) adrenergic receptor number and function in platelets; and 3) response to the agonist clonidine. Other approaches to understanding the pathophysiology of anxiety and its potential treatment with clonidine and carbamazepine will be explored.

PHS-6040
(Rev. 10-76)

I. Project Description

A. Objectives

This project employs a multidisciplinary team in the study and treatment of pathological anxiety and related mood disorders.

B. Methods Employed

1. Subjects

a. Patients who meet Research Diagnostic Criteria for panic, phobic, and generalized anxiety disorders are candidates for participation in the project. Patients are studied and treated on the 3-West Clinical Research Unit, Section on Psychobiology or through the Section's Outpatient Division. A number of previously validated scales to measure state and trait anxiety are utilized and an analogue anxiety scale has been developed to more adequately assess the relationship among state anxiety, phobic anxiety, and avoidance behavior.

b. Normal volunteers are also accepted into the project to provide control data as well as to assess the relationship between normal state anxiety and selected psychological and biological variables.

2. Psychological and Biological Evaluation

a. Baseline Evaluation. During an initial evaluative period patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation. This initial evaluation is indicated due to the heterogeneous nature of the panic and phobic disorders. Anecdotal reports suggest that many medical illnesses may present as or exacerbate pre-existing conditions of pathological anxiety. However, no research has systematically studied a large number of panic and phobic patients to determine the incidence and prevalence of these associated disorders.

b. Life Chart Methodology. A modified life chart technique has been developed to plot the frequency, intensity, and interval between panic attacks so that the development, recurrence, and progression of the panic and phobic disorders can be assessed. This is an important aspect of the project because few systematic studies have been conducted on the natural progression of these disorders.

c. Caffeine and Anxiety. In collaboration with Dr. J. P. Boulenger a caffeine inventory has been developed to assess the effects of caffeine on anxiety and related symptoms in panic anxious patients and normal volunteers.

d. Physiology. Motor activity is measured continuously every 15 minutes with a miniaturized activity monitor developed by Dr. T. Colburn. A number of investigators have demonstrated a relationship between state anxiety and noradrenergic-related substances (plasma MHPG, urinary MHPG, CSF MHPG) but the effects of motor activity on these measurements remain controversial. The

computerized activity monitor provides a unique method by which the relationship among these variables may be effectively investigated.

e. Sleep Research. Electroencephalographic sleep recordings are obtained for three consecutive nights. Although many panic anxious patients, like endogenously depressed individuals, have improved sleep following treatment with tricyclic and monoamine oxidase inhibitors, nothing is known about the sleep architecture of panic and phobic anxious patients. In collaboration with Dr. J.C. Gillin, this ongoing research represents the first attempt to our knowledge to evaluate the sleep profile of this patient population.

f. Insensitivity Index. Using threshold and signal detection methodology designed by Dr. M.S. Buchsbaum, an index of pain insensitivity is obtained in patients and normal volunteers following the intravenous administration of clonidine 2 µg/kg and placebo.

g. Galvanic Skin Response. The effects of clonidine and placebo on physiological measures of galvanic skin response, reaction time to auditory tones, pulse, and respiratory rate are studied in panic and phobic anxious patients and age-matched normal volunteers. This investigation is performed in collaboration with Dr. T. Zahn.

h. Echocardiography. Echocardiography is obtained in patients and age-matched controls to assess the presence or absence of mitral valve prolapse. These data are obtained in collaboration with Dr. R. Watson who is blind to the diagnosis of each patient or normal volunteer when echocardiography and auscultation are performed.

i. Clonidine -- An Alpha-Adrenergic Agonist. Clonidine is administered intravenously to anxious patients and volunteers to assess clinical, physiological, and neuroendocrine responses to this provocative noradrenergic challenge.

j. Urinary MHPG and Urinary Free Cortisol. Amine metabolites and urinary free cortisol are systematically evaluated using daily 24-hour urine collections across clinical state changes on and off medication.

k. Cerebrospinal Fluid and Plasma Studies. Amine metabolites, electrolytes, and peptides are also measured in blood and cerebrospinal fluid.

l. Alpha-Adrenergic Receptors. In collaboration with Dr. M. Kafka, platelet alpha receptor function as well as prostaglandin-stimulated increase in cyclic AMP are assessed in patients and age-matched normal volunteers.

m. Melatonin. Plasma and urinary melatonin is measured during clonidine and placebo infusions. Clonidine infusions will be administered to panic anxious patients and age-matched volunteers at night during sleep.

n. Glucose And Lactate Metabolism. Clinical and metabolic parameters are evaluated following the oral administration of 1.5 gm/kg glucose.

o. Dexamethasone Suppression Test. Dexamethasone is administered to patients to evaluate the pituitary adrenal axis. Basal values are performed at baseline and at 8 a.m., 4 p.m., and 11 p.m. following dexamethasone administration.

3. Treatment

a. Psychotherapeutic. Treatment and evaluation are conducted in individual supportive sessions. In addition, ongoing clinical case conferences are utilized.

b. Routine Somatic Treatment. Both routine and experimental compounds are evaluated during double-blind clinical trials. Standard medications used for the treatment of pathological anxiety may be used and include tricyclic antidepressants, monoamine oxidase inhibitors, minor tranquilizers, and beta-blockers.

c. Experimental Compounds. The anticonvulsant carbamazepine and the alpha-2 adrenergic agonist clonidine are in the preliminary stages of investigation as possible treatments of panic and phobic disorders.

C. Major Findings

This project has been active since November 1980. A number of noteworthy findings, however, have been elucidated during the six months since the initiation of this project.

1. Medical Illnesses and Pathological Anxiety

Detailed physical, neuropsychiatric, and laboratory evaluations have been performed in five patients who met Research Diagnostic Criteria for panic disorder. None of these patients had known pre-existing medical illnesses, yet within this group it has now been determined that three of the five patients have evidence of intracerebral pathology. One patient has a deep venous malformation in her right frontal-parietal hemisphere. One patient has complex-partial seizures and a discrete area of decreased uptake deep in her right cerebral hemisphere. One patient has a tremor and dystonic disorder of unknown etiology and a false positive VDRL. These preliminary findings are provocative and suggest the possibility of an increased incidence of and relationship between intracerebral pathology and some conditions of pathological anxiety.

2. Glucose and Lactate Metabolism

Four panic anxious patients have received a modified five-hour glucose tolerance test. Three patients (75%) developed anxiety and reactive hypoglycemia (glucose less than 55 MG/DL) 3½-4½ hours following the administration of glucose. These data may be of importance in understanding the relationship between glucose and lactate metabolism and state anxiety.

3. Anxiety and Psychophysical Pain

Preliminary studies in collaboration with Dr. M.S. Buchsbaum indicate a significant relationship between state anxiety and psychophysical pain ($r = .67$, $p < .02$). Our data suggest that the greater the state anxiety the greater the insensitivity to 93 randomly administered shocks 1 to 31 (mA) to the forearm. In addition, collaborative research with Drs. D.C. Jimerson and M.S. Buchsbaum indicate that pain sensitive individuals have low plasma free MHPG and low anxiety, and pain insensitive individuals have high MHPG and high anxiety. These findings, presented at the 1981 annual scientific proceedings of the American Psychiatric Association, represent one of the first reports that high anxiety may reduce the ability to discriminate the amount of pain a patient feels. These data further suggest that reducing a patient's anxiety with anxiolytics might intensify, rather than decrease, the amount of pain experienced as measured by the pain insensitivity index.

4. Clonidine as an Analgesic Agent

The potential use of the alpha-2 adrenergic agonist clonidine as a nonopioid, nonaddicting analgesic agent is of interest since clonidine has antinociceptive effects greater than or equal to morphine in animals and blocks and reverses opiate withdrawal in man. The acute effects of clonidine 2 µg/kg on psychophysical pain has been assessed in 14 normal volunteers. Using threshold and signal detection analysis as described by Dr. M.S. Buchsbaum, preliminary data indicate that clonidine lacks analgesic activity in man as measured by the index of pain insensitivity. However, there was a significant association between fall in blood pressure and increased insensitivity ($r = 0.71$, $n = 12$, $p < .01$). The fact that the subjects with the greatest fall in blood pressure had increased pain insensitivity may suggest that within individuals dose-response effects of clonidine may alter nociceptive thresholds in man.

5. Clonidine and Plasma Melatonin

The effect of clonidine on plasma melatonin during sleep has been studied in collaboration with Drs. A. Lewy and L. Siever. Clonidine 2 µg/kg i.v. produced at least a 50% reduction in plasma melatonin in all normal controls. This preliminary finding is noteworthy and provides a unique methodology by which noradrenergic responsivity in anxious patients may be assessed.

6. Clonidine as a Treatment for Anxiety

Clonidine was administered to 13 depressed patients and 12 normal volunteers to assess the effect of this drug on anxiety. Using the previously validated Spielberger State-Trait Anxiety Inventory (range 20-80), state anxiety was rated at baseline and one hour after clonidine or saline infusions. In depressed patients, ratings of anxiety significantly decreased after clonidine (50.2 ± 3.2) compared to baseline (59.8 ± 3.9) and did not change after placebo (pre 56.9 ± 4.6, post 58.6 ± 4.4). In normal volunteers anxiety following clonidine (pre 29.7 ± 1.6, post 30.7 ± 1.6) or saline (pre 30.4 ± 1.9, post 33.1 ± 2.3) did not differ from baseline. This differential antianxiety effect of clonidine in depressed patients and not in normal volunteers was highly significant (2-way ANOVAR, $p < .001$). These findings suggest that clonidine may have

noteworthy antianxiety effects in depressed patients as well as patients undergoing opiate withdrawal and support a role for noradrenergic mechanisms in the modulation of anxiety. It is unclear, however, whether this antianxiety effect is specific, is related to sedation, or can be maintained with chronic clonidine administration.

D. Proposed Course of Project

Studies of the clinical efficacy of clonidine and carbamazepine in panic and phobic patients have just been initiated. Our preliminary research with clonidine in depressed patients and normal volunteers is encouraging and suggests that clonidine might be especially useful in patients who experience context dependent panic attacks. In addition, carbamazepine will be studied with special emphasis on its potential usefulness as a prophylactic agent in blocking spontaneous panic attacks. These clinical trials, in conjunction with concomitant measurements of the neurotransmitter effects of clonidine and carbamazepine, should enhance our understanding of alterations in neurotransmitter pathways associated with pathological anxiety and its amelioration with appropriate psychopharmacotherapies.

It is hoped that the developing battery of clinical and biological tests in patients with anxiety and related disorders will ultimately provide a clinical and biological profile of these illnesses and lead to more refined subcategorizations, as well as to more selective and efficacious treatment approaches.

References

Uhde, T.W., Redmond, D.E., Jr., and Kleber, H.D.: Clonidine suppresses the opioid abstinence syndrome without clonidine-withdrawal symptoms: a blind inpatient study. Psychiatry Res. 2: 37-47, 1980.

Uhde, T.W., Post, R.M., Siever, L., and Buchsbaum, M.S.: Clonidine and psychophysical pain. Lancet 2: 1375, 1980.

Uhde, T.W., Post, R.M., Siever, L., Buchsbaum, M.S., Jimerson, D.C., Silberman, E.K., Murphy, D.L., and Bunney, W.E., Jr.: Clonidine: effects on mood, anxiety, and pain. Psychopharmacol. Bull., in press.

Siever, L.J., Insel, T.R., and Uhde, T.W.: Noradrenergic challenges in the affective disorders. J. Clin. Pharmacol., in press.

Uhde, T.W., Siever, L.J., and Post, R.M.: Clonidine: reflector of noradrenergic sensitivity in affective illness. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of Manic-Depressive Illness. Baltimore, Williams and Wilkins Company, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00072-01 BP

PERIOD COVERED
October 1, 1980 to September 1, 1981

TITLE OF PROJECT (80 characters or less)

Psychophysiological Investigation of Multiple Personality Disorder

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	E.K. Silberman, M.D.	Staff Psychiatrist	LPP NIMH
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2.0	2.0	0.0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study focuses on neurophysiologic and psychologic measures across the personality states of multiple personality disorder (MPD). In repeated randomized trials, subjects with MPD are tested to determine if reliable state differences in cognitive and physiological processing exist. Additionally, special psychological testing assesses the degree of memory overlap between states. Preliminary results suggest that the personality states differ significantly on a variety of psychological and biological measures. The existing literature is reviewed and a new questionnaire battery has been constructed to further assess the incidence, clinical presentation, etiological and treatment factors in this increasingly recognized and novel syndrome of the existence of multiple discrete personalities within the same individual.

PHS-6040
(Rev. 10-76)

I. Project Description

A. Objectives

This project is involved in a multidisciplinary effort to rigorously document and investigate the clinical phenomena observed in multiple personality disorder (MPD). There are two major avenues of investigative inquiry at the present time.

1. The first approach focuses on the study of the neuropsychological differences clinically observed to exist among the different personality states of an individual with MPD. This investigation emphasizes repeatable neurophysiologic measures which can be compared across personality states and contrasted with similar data from normals. In addition, specially designed psychological tests measure learning and memory within and across the personality states.

2. The second line of inquiry is an attempt to develop a large systematically collected data base on the incidence and prevalence of MPD in the United States. The emphasis of this study is on the presentation, diagnosis, clinical symptomatology, treatment, and outcome of this unusual disorder. The data for this study are derived from two sources: 1) a careful review of all cases in the American literature; and 2) a large scale questionnaire study directed at current cases in treatment across the United States.

B. Methods Employed

1. Subjects

a. Patients who meet DSM III criteria for MPD are admitted either as inpatients or outpatients to the 3-West Clinical Research Unit, Section on Psychobiology.

b. Normal volunteers and professional actors will serve as controls.

2. Neurophysiological Evaluation

a. Drug-free subjects undergo a series of visual averaged evoked potentials. Each personality state tested receives a trial of AER on five different days. At least three personality states are tested in each MPD subject.

b. EEG spectral power mapping studies, using the average of six runs to determine the spectral profile, are carried out.

The above studies are conducted in collaboration with Dr. M.S. Buchsbaum.

c. GSR, heart rate, pulse volume, skin temperature, and respiration are studied across personality states in collaboration with Dr. T. Zahn.

Each personality state is studied on five different days. An autonomic conditioning paradigm is used to measure learning and habituation across personality states.

d. Proactive inhibition memory testing developed by Drs. E.K. Silberman and H. Weingartner is used to measure the transfer of learning and memory across personality states and assess the clinically observed amnesia phenomena.

3. Questionnaire Study

An extensive computer-coded questionnaire derived from the clinical phenomenology noted in the systematic review of the literature has been prepared. This questionnaire covers the initial presentation of patients, method of diagnosis, past medical and psychiatric history, symptoms and phenomenology of the disorder, family and social history, and comparison of treatment modalities and outcome. The questionnaire will be initially distributed to two groups. The first group will be psychiatrists on the APA mailing list who return a postcard giving the number of cases they have seen and indicating a willingness to fill out the more extensive questionnaire. The second sample group will be a list of 500 clinicians who have treated one or more cases of MPD.

C. Major Findings

1. Neurophysiologic. Preliminary data analysis on three subjects who have completed the visual average evoked response study indicate that the differences between personality states within an individual reach statistical significance on several measures. Visual evoked potentials at four intensities were recorded during each personality state on four separate days. A 3-way ANOVA (subjects by personalities by intensities) revealed significant differences due to personality (subjects by personality interactions) for several components including the P100 amplitude at vertex ($F = 3.49$, 4,18 df, $p < 0.05$; changes in augmenting/reducing with personality type), as well as with some other components (e.g., P100 @ occiput, $F = 6.83$, $p < 0.05$).

2. Power spectral mapping studies, complete on one subject, demonstrate significant regional differences in EEG power distribution in some frequency bands. The different personality states appear to be topographically different, with some personality states showing more EEG activity in the temporal and occipital cortical areas than other personality states.

3. GSR and other autonomic measures are significantly different across the personality states, e.g., some personality states show as much as a 20 beats per minute difference in heart rate within the same individual. These autonomic differences were replicated on at least four randomized trials in three different individuals. Furthermore, autonomic measures of habituation to a stimulus in one personality state do not generalize to the other personality states within an individual.

4. The proactive inhibition memory paradigm demonstrates that while some exchange of memory does occur across personality states, the amount of information transfer is significantly decreased compared to normal controls.

Lists of words learned in one personality state are essentially limited to that personality state and are not accessible to other personality states within the same individual. Normal controls are unable to segregate the same word lists to a comparable degree, and words learned from one list intrude onto others, producing errors of commission.

5. A review of the literature on MPD reveals a consistency in symptoms and associated phenomenology over the last 150 years. The primary symptom is amnesia which is present in at least 80% of the cases reviewed. (Data taken from a subset of 22 well-documented cases). A large majority of these patients have a history of fugue states (77%) and most report a history of migraine-like headaches (68%). At least half of the patients had been previously diagnosed as schizophrenic; probably in part due to the frequent presence of persecutory auditory hallucinations (41%). Approximately one quarter of the cases reviewed had a history of seizures. A recent study, not included in the subset of 22 cases, revealed abnormal EEG's in 14 cases of MPD seen at Harvard (Mesulam, M.M., Arch. Neurol. 38: 176-181, 1981).

The phenomenology of the personality states also reveals certain consistent characteristics associated with the alternate personalities. More than half of the cases had a violent alternate personality who was responsible for a physical assault on one or more occasions. At least half of the cases had a depressed and suicidal alternate personality.

Childhood abuse, physical and sexual, seems to be a common historical feature in the majority of cases where data can be obtained. There is evidence that the dissociative splitting into alternate personalities occurs early in childhood and may be a consequence of this abuse.

The data derived from a thorough review of the literature indicate that MPD is a syndrome with consistent symptoms and phenomenology. The behavior of the alternate personalities frequently brings the individual into contact with legal and medical systems. Presently, there is no controlled data available on the effectiveness of various therapeutic interventions. The possible etiologic effect of child abuse needs to be further investigated. Possible early detection may aid in prevention of further dissociation and aid in more rapid integration of the individual.

D. Implications of the Research

The preliminary physiological data suggests that the clinical observations and patient subjective reports of profound psychological and physiological differences between personality states are valid. The physiologic differences among the states are replicable on repeated trials in the same individual and the personality state differences have been observed to a statistically significant degree in all three of the individuals who have completed the protocol.

The proactive inhibition memory testing has demonstrated in the three individuals a separation of learning and memory between personality states within a given individual. This tends to confirm the subjective reports of individuals with MPD that the experiences and memories of one personality state

are separate from other personality states. Taken together these two lines of evidence suggest that a discrete psychologic state may have specific physiologic concomitants.

Multiple personality disorder appears to be a far more common syndrome than previously recognized. The emerging data from the physiologic studies and the possible etiologic nature of child abuse have potentially important social and legal ramifications for recognition and prevention of this unusual disorder.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z0L 00049-07 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Psychosocial Factors in the Prognosis of Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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0.8	0.6	0.2

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☐ (a1) MINORS ☒(a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the effect of various psychosocial factors on host resistence to malignant melanoma. A detailed characterization of these factors in patients with melanoma, Clinical Stage II, is being obtained. In addition, certain endocrine and immune parameters are also being studied. We are testing several hypotheses which may distinguish those patients suffering recurrence of their disease from others who either remain disease-free or have a delayed recurrence. At one year, significant correlations with disease-free status were found with (1) number of positive lymph nodes, (2) ABO type, (3) social assets and (4) melanoma adjustment self-rating score.

In a second group of 30 melanoma patients, findings (1) and (4) were replicated as predictors of relapse within one year.

PHS-6040
(Rev. 10-76)

Project Description

Objectives

This study is aimed at developing a detailed characterization of the psychosocial factors which distinguish those patients who suffer recurrence of malignancy from those who remain disease-free or who have a delayed recurrence. Data is being gathered to test several current hypotheses concerning psychosocial antecedents of the onset of physical illness and predisposing psychobiological states.

Fifty to 100 patients with "surgically-cured" Stage II Melanoma will be studied. We plan a multi-level assessment of each patient. In addition to the biological measures gathered on the patient, we will characterize the patient's genetic history, past psychosocial history, level of personality development, current life stress and present psychological state. We will accomplish this by (1) psychiatric interview, (2) objective assessment of a five-minute speech sample, and (3) standardized questionnaires including MMPI, Holmes and Rahe Schedule of Recent Experience, SCL-90, Rotter Internal-External Locus of Control Scale, Beck Depression Inventory and the Gottschalk In-Out Scale.

The patients are seen shortly after their "curative" surgery and will be followed up at 2-month intervals, at which time we will obtain the Holmes-Rahe Scale, the SCL-90, Helplessness-Hopelessness Index and Beck Depression Inventory.

At one year, significant correlations with disease-free status were found with (1) number of positive lymph nodes, (2) ABO types, (3) social assets and (4) melanoma adjustment self-rating score. We are presently examining this finding in a third group of patients. The ABO finding did not hold up but the melanoma adjustment score did.

Data analysis is underway.

Publications:

None

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PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Biological and psychopharmacological evaluation of schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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14	11	3

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the United States alone, it is estimated that over 2 million people suffer from schizophrenia. Because of its very nature, this disorder is difficult to study. Pharmacological treatment is a particularly effective intervention in schizophrenia but is not a cure for all patients. Experimental and clinical findings have indicated that schizophrenia is a heterogeneous psychiatric disorder with biochemical, psychophysiological, psychological and genetic components. Patients are not psychotic constantly and their clinical state may change, regardless of medication conditions. However, we are now able to study a schizophrenic patient population intensively and longitudinally, with and without pharmacological approaches during different clinical states. We are beginning to ask fundamental questions about the nature of schizophrenia, indications for pharmacological treatment, vulnerability to psychotic decompensation and the role that genetics may play in the transmission of the disorder.

Our work integrates the multidisciplinary approaches in an effort to understand this complex disorder which may lead to a more effective treatment.

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Project Description

The Section on Neuropsychopharmacology (1) plans and conducts multidisciplinary research on the etiology and treatment of schizophrenia and (2) conducts research to define the biological dysfunction in schizophrenia.

Objectives

1. Multidisciplinary research on the etiology, treatment and prevention of schizophrenia.
2. Evaluation of the dopamine, other amine and peptide hypotheses of schizophrenia
3. Evaluation of potential markers of vulnerability and predictors of antipsychotic drug response
4. Psychopharmacological and biochemical evaluation of trait- and state-dependent variables of schizophrenia
5. Evaluation of novel treatments such as hemodialysis, propranolol and naltrexone.

Methodology

One to 14 days after admission in a double-blind fashion, patients are taken off their neuroleptic medication and put on placebo. After this 2-week "washout period," patients go through a 3 to 4-week test period in which diagnostic evaluation, medical and neurological workup, cerebrospinal fluid (CSF) collections, drug infusions (amphetamine, beta-endorphin, placebo), psychological and psychophysiological testing and neuroendocrine evaluations take place. These are followed by two or more drug trials which include pimozide and propranolol. During any of these drug trials, testing similar to the admission test week can take place. Each drug period is separated by a 2-week placebo "buffer" period. The last of these periods is either followed by another test period or immediately followed by a 4-week clinial period where, if indicated, the patient is put on generally available antipsychotic medication and prepared for discharge. This extensive data-collection system allows us to evaluate the individual variables and drug responses against one another to observe the patient in different clinical states. It enables us to develop psychosocial, endocrine, biochemical and pharmacological profiles on patient groups defined by each variable.

A major problem of schizophrenia research has always been certainty of diagnosis. In addition to using the International Pilot Study of Schizophrenia criteria as developed by the World Health Organization and the former Psychiatric Assessment Section of the Adult Psychiatry Branch, we are applying the Research Diagnostic Criteria (RDC) as described by Dr. Spitzer and his collaborators to define diagnostically our patient population. This coming year, RDC will be replaced by somewhat more descriptive DSM III criteria.

The 4-East Nursing Staff has been trained in the application of the Bunney-Hamburg psychosis, depression and mania scale and we have added a scale to

measure extrapyramidal side effects (EPS) and sedation. The Brief Psychiatric Rating Scale modified by the NIMH behavior rating scale committee under Dr. D. L. Murphy was adopted for biweekly ratings by our nursing staff and is used weekly by "blind" psychiatrist raters.

Hemodialysis of Schizophrenia

Eight patients completed a 20-week hemodialysis trial (10 real and 10 sham) in a double-blind evaluation of the potential for this experimental procedure. None of the patients improved. A special cartridge was developed to maintain the double-blind. Patients underwent drug-free testing, including CSF studies, endocrine and psychophysiological testing (Drs. Balow, Schulz, Bunney).

This project stretched the expertise of psychiatrists and nursing staff alike because it required severely ill psychotic patients to remain engaged in the project for up to 6 months without intervention of antipsychotic agents. Mrs. S. Beal and her staff are to be complimented for their collaboration and support. This study was the most difficult we ever undertook because of the long duration of drug-free management and the fistula operation for blood access.

We received over 150 requests to participate in this protocol: 23 patients were admitted for evaluation and 11 patients started the protocol. Although our report is completely negative, further double-blind evaluation of hemodialysis at other facilities may be needed to make a definitive statement. At this time there is no evidence that hemodialysis is indicated as a treatment for schizophrenia. The final report of the study was published in Science 211:1066-1068, 1981.

Data from studies carried out in collaboration with Dr. Buchsbaum (Positron Emission Testing Scan, AER, pain testing), Dr. Gillin (sleep), Dr. Weingartner (cognitive function), Dr. Pickar (beta-endorphin), Dr. Zahn (psychophysiology) and Dr. Gold (spinal fluid neuropeptides) are being analyzed.

Presently we are studying plasmapheresis as a means of testing the role of an "endogenous" toxin or immunoglobulins in schizophrenia. Immunoglobulins are reduced for 90% of preplasmapheresis values. The collaborators are Dr. Klein, Clinical Center Blood Bank Department and Dr. Papadopoulos, Clinical Pathology Department, Clinical Center.

New Assays and Drugs

In collaboration with the Section on Biochemistry, BPB and the Clinical Psychobiology Branch, new assays for measuring peptides in spinal fluid have been started. We presently have drugs available that are relatively specific for the different neurotransmitter systems that we are studying.

Major Findings: Pharmacological

A. Dopamine Stimulation (d-Amphetamine)

d-Amphetamine, an indirect dopamine receptor agonist, has been shown to worsen some acute psychotic symptoms. At the same time, numerous clinical reports of increased social contact and therapeutic effects have appeared in the psychiatric literature over the last 25 years. This, therefore, seems a potentially crucial area of investigation for clarifying the limits and nuances of the dopamine hypothesis. Patients receive i.v. amphetamine and i.v. placebo during the initial test-period. This is repeated during pimozide, pimozide withdrawal and lithium treatment.

A standardized rating system has been developed for quantitating behavioral change before, during and after the infusion interview. Additionally, blood samples are taken regularly during the day to monitor amphetamine blood levels, serum prolactin, norepinephrine and DBH levels. Clinically, schizophrenic patients appear to respond differently to amphetamine than do depressed patients, although there is heterogeneity within each group. (Several papers are in preparation or have been submitted.) Strikingly, depressed patients do not become more psychotic; only 18 of 45 schizophrenic patients showed increased psychotic symptoms. However, within this schizophrenic group the more severely psychotic patients may improve, while some patients in early remission may get worse. Preinfusion spinal fluid MHPG correlated significantly with the change in psychosis. Neither chronic pimozide (neuroleptic) pretreatment nor lithium attenuated the behavioral effects of amphetamine. No increased behavioral response was observed following amphetamine administration during the pimozide withdrawal phase (DA receptor supersensitivity). We replicated our previous finding that prolactin levels were increased 25 and 40 min. postamphetamine infusion. This was rather striking since the placebo infusions which took place at the same time of the day--between 8-9 a.m.--showed a slight decrease from the rise of prolactin during sleep as a result of diurnal variation. This is in contrast to the expected decrease in prolactin levels following increased dopamine activity. The increase is hypothesized to be due to stress or activation of serotonin mechanisms in the hypothalamus and pituitary. The principal investigator was invited to the Gordon Conference for Catecholamines in Andover, New Hampshire to present the d-amphetamine studies.

B. Dopamine Infusion

In collaboration with Drs. Zis, Gold, Yen and Dr. DeFraites, DA infusions were used to test the prolactin, growth hormone (GH), luteinizing hormone (LH) and follicle-stimulating hormone in schizophrenic patients during drug-free, neuroleptic-medicated and neuroleptic-withdrawal phases. DA does not cross the blood-brain barrier and acts, presumably, at the DA terminals of the pituitary and median eminence. Preliminary data indicate that schizophrenic patients may have an abnormal LH response and a supersensitive prolactin

response to DA in the neuroleptic-withdrawal phase. Dr. DeFraites presented this data at the American Psychiatric Association Meeting, New Research Session (New Orleans, 1981).

C. Dopamine Receptor Blockade and Schizophrenia

Pimozide in acutely psychotic patients: following a washout period, patients are started in a double-blind, placebo-controlled trial of the effects of pimozide in acutely psychotic and remitted patients. This antipsychotic agent is of special interest because of its relatively specific postsynaptic dopamine receptor blocking effect.

During this project endocrine studies and CSF studies are conducted. Selected patients are put on sleep study to evaluate the effects on sleep. This is in contrast to the behavioral effects of amphetamine, which are reversed in those who improve while drug-free after amphetamine and who then become more psychotic after pimozide. We also gave amphetamine infusions seven days after pimozide discontinuation to evaluate whether receptor hypersensitivity is connected with a change in behavioral response. Data from this project are presently being analyzed. However, we did not observe a stronger response to amphetamine, which questions the dopamine hypersensitivity hypothesis of psychosis. A paper evaluating the effects of pimozide on sleep in psychiatric patients has been published, as well as a paper on the pimozide attenuation of the amphetamine-induced REM suppression.

Other studies include endocrine studies, cyclic AMP in CSF, lack of effect of pimozide on DBH in spinal fluid, metabolites of dopamine, norepinephrine (decrease) and serotonin in the spinal fluid and 24-hour urine collections, in addition to the standard battery of behavioral ratings. In collaboration with Dr. Heykants of Beerse, Belgium, we are measuring pimozide levels in our patients with a radioimmunoassay. These levels will be examined in relationship to the above-mentioned hormones and urinary free cortisol. We are now exploring CSF and endocrine variables at different time-points of the pimozide trial, including during the withdrawal phase.

D. Lithium in Schizophrenia

Since lithium attenuates some of the behavioral activation induced by amphetamine, lithium may have some antidopamine effects. We studied lithium carbonate in psychotic and remitted schizophrenic patients: nine of 13 psychotic patients responded with an improvement either in psychosis or depression. Diagnostic categories did not separate the responders from the nonresponders. Six of 11 remitted patients who showed postpsychotic depression improved on lithium. We are now studying biochemical and other biological variables to determine which of these may predict a response to lithium. One paper has been published and one paper is in press. Response to d-amphetamine appeared to correlate with antidepressant and antipsychotic response to lithium carbonate treatment for three weeks. This study needs replication.

The data were presented at the APA (New Orleans). Lithium in schizophrenia, a review of the literature and discussion of our own data was presented at the APA in New Orleans also.

E. Gamma-Aminobutyric Acid and the Dopamine Hypothesis of Schizophrenia

(a) GABA levels in CSF of schizophrenic patients are presently being studied in collaboration with Dr. Hare. In 50 schizophrenic patients GABA levels were not significantly lower than in age-matched female controls. Levels increased with duration of illness, number of hospitalizations and months of hospitalization. This suggests that with time the low levels are being compensated.

(b) Diazepam, one of the first compounds found to increase GABA function in the brain, was tried on our patients. We administered diazepam in doses higher than 40 mg per day to five patients. Only two severely psychotic patients received 300 mg of diazepam with equivocal results. One patient, who was still relatively nonpsychotic following pimozide withdrawal, became acutely psychotic when he was withdrawn from 70 mg of diazepam. The more psychotic the patient, the fewer the side effects there were like ataxia and sedation. A paper has been submitted by Dr. Jimerson.

(c) A paper about the effect of gamma-hydroxybutyrate (GHB) in schizophrenia has been accepted for publication (Dr. Schulz). A GHB-associated improvement was observed in two patients. In general, GHB activated schizophrenic symptoms. A hint that low platelet MAO, high CSF HVA and an augmentation with AER technique suggested improvement was present. The two patients improved the most after withdrawal of GHB. This project has ended.

F. Endocrinological Project

Endocrine studies remain a major investment of the Section.

(a) Evaluation of baseline hormonal levels in schizophrenic patients is being conducted, focusing on hormones that are under total or partial control of DA. Prolactin, GH, FSH and LH are measured in serum. Measurements thus far have been made with placebo, chronic pimozide, GHB, propranolol and naltrexone. Increase in prolactin levels followed DA blockade (by pimozide) as expected but no effects were observed with (chronic) propranolol (for the males).

(b) We also studied the GH and prolactin response to apomorphine in drug-free psychotic patients. Patients participated three days after neuroleptic withdrawal, three weeks after placebo and one day after GHB withdrawal. These data are presently under analysis.

(c) Urinary-free cortisol (UFC) levels in drug-free patients and in patients on opiate and pimozide and on the antagonist, naltrexone, are being examined. In contrast to the report by Drs. Chen and Carrol, some schizophrenic patients do seem to have increased levels (like depressed patients). This has led us to study the dexamethasone suppression test early in the psychotic episode.

Naltrexone had no effect. Pimozide seemed to normalize UFC levels. During pimozide withdrawal a further significant decrease in UFC was observed (paper in preparation).

(d) Chronic pimozide treatment increases serum prolactin and decreases LH. A paper has been published.

(e) Hypertonic saline infusions have been started in drug-free and medicated patients (Drs. Malas and Gold).

(f) Amphetamine infusion did not change plasma radioimmune beta-endorphins (with Drs. Schulz and Pickar).

G. Cerebrospinal Fluid Studies (CSF) in Schizophrenia

Baseline and probenecid-accumulated metabolites of DA, NE, serotonin, tyramine and phenethylamine in CSF are being measured with mass spectrography.

Early evaluation of pimozide's effect on HVA showed that eight responders had a subsequent rise of HVA but three nonresponders had a decrease. Further patient data have not supported this early observation.

Measurement of viral antibody titers in CSF has been temporarily discontinued.

CSF studies for c-AMP and c-GMP mean values were not different from depressed comparison groups of patients. Probenecid increased the baseline values. The effects of pimozide are presently under study.

A study of pterine cofactor activity in our schizophrenic patients and in normal comparison groups was started (J.C. Garbutt, R. Levine, W. Lovenberg and J. Ballenger). Schizophrenic patients had similar levels to those of 20 normal controls. A paper has been submitted.

Evaluation of peptides such as vasopressin, vasotocin, oxytocin, angiotensin I and II, somatostatin and endorphin-like activity in CSF has been started.

For evaluation of CSF GABA see above; for CSF NE, DBH and endorphins see below.

H. Norepinephrine (NE)

(a) Dr. Marian Kafka measured alpha-receptor activity in our drug-free patients. Although the alpha-receptor activity is not different in schizophrenic patients compared to normal controls, 10% of the patients had very high levels. In the male patients c-AMP production was 50% lower than in the controls in response to prostaglandin stimulation. This decrease seems to be related to decreased activity in adenylcyclase. Ten percent of the schizophrenic patients appeared to have increased $alpha_2$-receptor binding in

platelets. She presented this data at the APA meeting in New Orleans. Further studies are underway.

(b) Dr. Tallman measured beta-endorphin receptors on leukocytes from psychotic and nonpsychotic patients during drug-free treatment and during high dose d- and dl-propranolol administration. There appears to be no difference between schizophrenic patients and normal controls while the receptor count stays stable over time.

(c) DBH in CSF of schizophrenic patients showed similar levels to that of normals but good premorbid (reactive patients) appeared to have lower levels. Patients on pimozide improved in psychosis relative to the decline in CSF NE ($r = .71$, $p < .02$). A paper has been accepted for publication. Dr. van Kammen presented these data at a CSF symposium at the University de Austral, Valdivia, Chile, December 1980.

(d) The clinical evaluation of d- and dl-propranolol has been discontinued with concomitant measurement of plasma levels, beta-receptor binding, endocrines and CSF metabolites. The data analysis is under way.

I. Endorphins

(a) In collaboration with Drs. Schulz, Wagner, Pickar, Rogol, Davis and Li, we reported on our studies with beta-endorphin infusions to schizophrenic patients for behavioral and endocrinological evaluation. Prolactin was raised by beta-endorphin in doses between 10-15 mg but behavior was not influenced.

(b) A study with a longer-acting opiate antagonist, naltrexone, has been completed. Initial results have been reported by Dr. Davis. Three of five patients improved. Pain-tolerant schizophrenic patients showed reversal of this tolerance with naltrexone.

(c) Our drug-free patients appeared to have low or normal radioimmune beta-endorphin and radio-receptor opioid activity in the CSF.

Major Findings: Physiological - Anatomical

A. Laterality Hypothesis

All patients are tested with the Luria test for lateralized sensory and motor function. Data will be compared to drug response and CAT SCAN abnormalities (Lee Mann).

B. CAT SCAN abnormalities have been found in about 60% of our patient population, independent of drug use and duration of illness. Psychological and biological exploration of this finding is underway.

C. Positron Emission Scans: This new method of evaluating brain function has been started in eight drug-free patients with Drs. Buchsbaum, Ingvar and Kessler. Papers have been presented at the APA in New Orleans and at the World Biological Psychiatry Meeting in Stockholm, Sweden.

D. EEG Recording of Sleep

Hemodialysis does not seem to affect sleep in our patients. With Drs. Mendelson, Gillin and Buchsbaum we have started 16 lead EEG sleep recordings to "map" the EEG frequency in relation to underlying brain structure.

E. Psychophysiological Testing

(a) Dr. Zahn of the Laboratory of Psychology and Psychopathology is testing our patients during drug-free treatment as well as during different drug conditions.
(b) Pain testing and average evoked response (AER) potential studies are being conducted by Dr. Buchsbaum.

Major Findings: Behavioral and Psychological

A. Drug Withdrawal

We observed that eight of 24 psychotic patients, whom we were not able to differentiate clinically on admission, remitted when their antipsychotic medication was withdrawn over a 30-day period. Although these patients all had been diagnosed schizoaffective-schizophrenic according to RDC classification (1975), this diagnosis did not differentiate the drug-free remitters from the schizoaffective nonremitters. The drug withdrawal improvers appeared to have a later age of onset, a shorter time of hospitalization and better premorbid functioning. The drug-free improved male patients had low platelet MAO activity below the mean for normals, while the nonimproved patients had platelet MAO activity ranging from high to low. The drug-free improvers also responded to lower than the suggested dose ranges for the nondrug-free improvers. This continued to be present at one-year follow-up (150 vs. 484 clorpromazine units/day). This finding may lead to a differential approach of medicating acutely psychotic patients. It suggests that if acutely schizophrenic patients do not improve on neuroleptic drugs at 4-6 weeks, the dose may need to be decreased rather than raised in some patients. The phenomenon of brief improvements during drug withdrawal will be explored further.

B. Hemodialysis

Eight patients have finished 20 dialyses each that included 10 active ones; none of the patients improved and four worsened. Three papers have been published.

C. Information-processing Project

(a) A simply administered, objective, quantified measure of cognitive disorder has been developed. Preliminary results have disclosed a highly significant difference between patients and normals. We will use this technique to investigate the effects of psychoactive drugs and state variables on schizophrenic thought disorder. We are also investigating the presence and degree of thought disorder in the families of schizophrenic patients which appeared to be present in at least one parent. A paper has been accepted for publication.

(b) Schizophrenic thought disorder is also being investigated with a battery of psychological tests mainly derived from standard I.Q. tests; some scores appear to be impaired in schizophrenia. This emphasizes the need for a special psychological evaluation of our patients.

(c) Evaluation of memory and cognition in schizophrenia are underway by Dr. Weingartner.

Major Findings: Genetic

A. HL-A Typing in Acute Schizophrenia

HL-A antigens are genetically determined human cell membrane antigens found on a variety of cell types. The genes controlling their expression are found on chromosome 6 and are thought to be linked closely to genes controlling immune response. In recent years, associations of certain HL-A types and certain diseases have been found. These diseases are frequently those associated with with causal disorders of immunity, possibly the result of aberrant immune-response genes--thus their association with HL-A.

Schizophrenia may have causal or secondary immune abnormalities; therefore, a study of HL-A antigen frequencies in this disease seemed worthwhile. If found, this would constitute more evidence for the role of the immune system in schizophrenia. To date we have typed 75 schizophrenic patients.

C. Monoamine Oxidase (MAO)

Monoamine oxidase (MAO) in plasma or platelets has been implicated in vulnerability to chronic unremitted schizophrenia and affective disorders. We are presently evaluating clinical and biochemical variables to activity levels. Preliminary data in our male drug-free remitted schizophrenic patients indicate lower than the mean compared to a normal control population. A brief communication has been published. Our patients appear to have significantly lower MAO activity than normal controls. CSF NE correlated significantly negative with MAO activity.

Significance to Mental Health Research

These studies contribute to the understanding of schizophrenia. Our work integrates the multidisciplinary approaches to this disorder.

Hemodialysis: The claims of miraculous cures (1977) are under investigation throughout the world. To date (May 1981) there are many places in this country where hemodialysis is already being used as a treatment for schizophrenic patients. Our double- blind evaluation study did not show improvement in our patients. Dialysis for schizophrenic patients should remain an experimental procedure until other double-blind studies indicate definite improvers. Plasmapheresis will be a better test of the therapeutic claims of hemodialysis and may help to understand the hypothesized immunological disturbance in schizophrenia.

Lithium can be of help in the management of schizophrenic patients, although it is less effective than neuroleptics. It may have antidepressant properties in postpsychotic depression. Amphetamine effects may predict these drug responses in schizophrenia patients.

Serum and CSF DHB was lower in some good premorbid, neuroleptic-responsive schizophrenic patients. CSF NE was higher than in normal controls but decreased with neuroleptic-induced improvement. These findings do not support the Stein and Weiss hypothesis of schizophrenia. However, NE correlated with the age of onset in female patients.

Prostaglandin-stimulated cAMP formation is less in schizophrenic males and females compared to normals. This may implicate prostaglandin in schizophrenia. Alpha-receptor binding was increased in 10 schizophrenic drug-free patients.

Schizophrenic patients as a group appeared to be more pain tolerant than normals. This could be reversed by naltrexone, which may imply a disturbance of endorphin regulation of pain mechanism in schizophrenia. CSF endorphins (radioreceptor and radioimmune assay) were not elevated in our schizophrenic patients.

The amphetamine-induced improvement in psychosis and the relative lack of worsening during the pimozide-withdrawal-phase amphetamine infusion raised questions about generalizing the DA hypothesis of schizophrenia and suggested that DA subsensitivity may be present in chronic schizophrenia. We observed that amphetamine-induced worsening during pimozide treatment predicted early relapse after pimozide withdrawal. Lithium pretreatment decreased the activating effects of d-amphetamine but chronic pimozide pretreatment did not. Our findings support the notion of state-time dependent vulnerability to psychotic decompensation which is only partially decreased with neuroleptic treatment. The d-amphetamine infusion test during neuroleptic treatment may have similar implications for neuroleptic treatment withdrawal as the dexamethasone suppression test in affective illness for discontinuation of tricyclic antidepressants.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00081-07 BP

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Heritable Characteristics of Cation Transport in Primary Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John I. Nurnberger, Jr.,M.D.	Senior Staff Fellow	BP	NIMH
OTHER:	E. S. Gershon, M.D.	Chief, Section on Psychogenetics	BP	NIMH
	D. C. Jimerson, M.D.	Staff Psychiatrist	BP	NIMH
	Sue Simmons, M.S.N.	Clinical Nurse Expert	CC	NURS

COOPERATING UNITS (if any)
Illinois State Psychiatric Institute, Chicago, Illinois; University of Chicago

LAB/BRANCH
Biological Psychiatry Branch

SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is the identification of heritable characteristics of ion transport which may distinguish individuals with a primary affective disorder from normal controls.

A study of sodium potassium stimulated adenosine triphosphatase (Na^+ -K^+-ATPase), a cation transport enzyme has been completed and data is being analyzed. We have reported that Nak ATPase is a "state" but not a "trait" marker for affective illness. We find now that plasma cortisol is negatively correlated with Nak ATPase in patients with affective disorder.

We are engaged in a collaborative program of research on the lithium erythrocyte/plasma ratio in euthymic patients. We previously confirmed that bipolar patients have higher ratios than controls. However, we have noted an extended effect of lithium treatment on the ratio and are doing further studies to delineate this variable.

PHS-6040
(Rev. 10-76)

Project Description

A spectrophotometric assay has been used to determine levels of $Na^+ - K^+$ - ATPase in human erythrocytes. We have previously found that Nak ATPase is a "state" but not a "trait" marker in affective disorder (only during episodes of depression is the level decreased.) We recently found a negative correlation between Nak ATPase and plasma cortisol ($r = -0.31$, $p < .02$) in our population. Cortisol itself is higher in euthymic patients than in controls and thus may be a "trait" marker.

We are examining the response of the enzyme to arecoline stimulation, since arecoline has induced depressive symptoms in some subjects. Arecoline reduces the Nak ATPase activity in the data analyzed from eight subjects.

Lithium transport parameters have been extensively studied by Pandey and colleagues at the Illinois State Psychiatric Institute. We have entered into a collaboration with them in an attempt to replicate their finding that lithium erythrocyte/plasma ratio is a genetic marker for affective disorder. Preliminary results have confirmed that patients have higher ratios than controls ($p < .02$). We have collected 31 further samples to investigate the relationship of the ratio to time off lithium, and hope for results on this question soon. If we confirm the findings of Pandey and colleagues, we will pursue a study of relatives of bipolar patients. Eleven patients and nine normal volunteers participated in these studies.

Significance to Mental Health Research

Cation transport mechanisms depend on cell membrane characteristics that are likely to be largely genetically determined. Previous studies have demonstrated differences in cation transport mechanisms in affectively ill patients. We have demonstrated an inverse relationship between cortisol and ATPase in patients with affective disorder, providing new evidence about physiological mechanisms in this illness. As part of pharmacologic challenge strategy, Nak ATPase activity may differentiate patients from controls.

The finding that lithium erythrocyte/plasma ratio acts as a genetic vulnerability factor in pedigrees of people with affective disorder is extremely important to confirm or disprove. Such a factor would be important in predicting illness within families as well as understanding the etiology of affective disorder.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00082-07 BP

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Life Events, Family Attitudes, and Marital Dysfunction in Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. D. Dibble, D.S.W.	Psychiatric Social Worker	BP	NIMH
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	S. Simmons, M.S.N.	Clinical Nurse Expert	CC	NURS

COOPERATING UNITS (if any)
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been terminated, with two publications.

PHS-6040
(Rev. 10-76)

Publications:

1. Targum, S.D., Dibble, E.D., Davenport, Y.B., and Gershon, E.S.: The family attitudes questionnaire: Patients' and spouses' views of Bipolar illness. Arch. Gen. Psychiatry 38: 562-568, 1981.

2. Frank, E., Targum, S.D., Gershon, E.S., Anderson, C., Stewart, B.D., Ketchum, K.L., Kupfer, D.J.: A comparison of "normal" and bipolar-well spouse couples. Am. J. of Psychiatry. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00084-07 BP

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Genetic-Biologic Studies of Psychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
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6.8	5.0	1.8

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☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Family Study of Affective Disorders: The transmission of several affective illnesses in first degree relatives fits a multifactorial-inheritance model of a single underlying liability. Schizoaffective illness represents the most extreme liability, next is bipolar, and unipolar illness has the smallest genetic liability. Single-locus models do not fit these data. Dopamine-beta-hydroxylase in plasma is linked to the ABO blood group locus on chromosome 9, but is unlinked to affective illness. A cell culture laboratory with a series of fibroblast cell lines from patients and relatives was established. The fibroblasts show specific binding suggestive of muscarinic cholinergic and beta-adrenergic receptors. Genetic variation in plasma methoxyhydroxyphenylglycol (MHPG), plasma gamma-hydroxy butyric acid-(GABA), platelet ^{3}H-imipramine binding, and platelet GABA-transaminase, have been identified.

OTHER INVESTIGATORS:

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D. Pickar, M.D.	Chief, Unit on Studies of Drug Abuse	BP	NIMH
R. Post, M.D.	Chief, Section on Psychobiology	BP	NIMH
I. Kopin, M.D.	Chief, Laboratory of Clinical Sciences	LCS	NIMH
F. Goodwin, M.D.	Chief, Clinical Psychobiology Branch	CPB	NIMH
M. Kafka, Ph.D.	Research Psychologist	BP	NIMH

Project Description:

1. Family Study of Affective Illness.

This year additional data on schizoaffective disorder was collected but the greatest amount of effort has been on data analysis, by us and by our collaborators at Yale headed by Dr. Myrna Weissman. Reliability between the two centers was established by exchange of personnel and by each team interviewing subjects separately at different times.

Some methodological findings have emerged. A comparison was made between data collected by a family history and retrieval of medical records, and direct interview data. In our data, the same frequency of illness appears in relatives divided into three types of information source (relative directly interviewed, relative not interviewed but information from multiple sources, relative not interviewed and only source is the patient.) The reason for this consistency in our data appears to be the low rate of refusal of patients to have their relatives contacted, and a high rate of interviews of relatives (four immediate relatives or spouse per patient). In New Haven, there was a reduced prevalence in relatives where the proband did not give permission to have his relatives contacted and this was a considerable portion of their data. If we correct for this discrepancy statistically, there is the same frequency of illness in relatives of the one class of patients studied in both places (Unipolar patients with hospitalization).

The New Haven data and our data both show higher prevalences in relatives than some previous studies, even when the same diagnostic methods and criteria were used (such as Dr. Gershon's previous study in Israel). The reason appears to be differences in the culture of perception of psychological illness. This explanation is supported within our own data: there appear to be cultural effects on reported diagnoses in uncles, aunts and grandparents, as opposed to sisters and parents. This appears to be a generational effect. The higher rate of suicide with no previous psychiatric illness in the older generation, and the decreased incidence of affective illness and of affective illness as a precursor to suicide, imply differences between the different generations in recognizing affective illness, according to our interview and diagnostic criteria.

	Suicide as First Diagnosis		Suicide with Preceding Diagnosis of Affective Disorder	
Relative type:	First degree	Second degree	First degree	Second degree
Suicide	2	15	13	2
Other	987	1379	976	1392

The difference between the first and second degree relatives in both cases is highly significant statistically. The implication is that the affective illness phenotype is not precisely definable clinically. However, studies of diagnostic prevalence in the same population, with contemporaneous subjects, using the same criteria and methods of interview, are reproducible, since the New Haven data are close to our own.

Another methodologic point has to do with specificity and sensitivity of diagnosis.

Comparison of Interview Diagnosis with All Sources Diagnosis
(Includes Interview, Medical Records and Family History)

All Sources Diagnosis	Interview Diagnosis					
	S-A	BPI	BPII	UP	Other	Total
Schizoaffective (S-A)	10	3	1	5	4	23
Bipolar I (BPI)	1	111	8	6	2	128
Bipolar II (BPII	0	2	52	15	2	71
Unipolar (UP)	0	1	0	143	13	157

As seen from the table, the Bipolar I, Bipolar II and Unipolar patients and relatives gave reasonably specific descriptions of themselves, whereas the schizoaffective patients less frequently described themselves as schizoaffective.

Prevalences in Relatives: Age corrected prevalences on 989 adult parents, sibling and offspring were:

First Degree Relatives

Diagnosis of Patient	Diagnosis of Relatives, %				
	S-A	BP I	BP II	UP	N
Schizoaffective (S-A)	6.2	10.9	6.2	14.7	83
Bipolar I (BP I)	1.1	4.5	4.1	14.0	548
Bipolar II (BP II)	0.6	2.6	4.5	17.3	191
Unipolar (UP)	0.7	1.4	1.4	16.6	166

A multiple threshold model of multifactorial inheritance satisfactorily accounts for these data, where the liability threshold to schizoaffective disorder is highest (furthest from population mean) the thresholds for BP I and BP II are intermediate (set equal to each other) and the threshold for Unipolar illness is lowest. In this model, there is noteworthy correspondence of clinical severity of each form of illness with its transmission liability.

Single locus autosomal and X-chromosome models were applied to the extended pedigrees of these patients. These were consistently rejected or indistinguishable from non-genetic transmission.

Affective illness in offspring of patients is considerably more prevalent than previously thought. If one parent has any of the major affective disorders (Schizoaffective, Bipolar or Unipolar) the lifetime prevalence in offspring is 27%. If both parents are ill, the prevalence is estimated at 57%.

Other factors in the patient such as age of onset, multiple episodes, or presence of alcoholism do not affect the morbid risk to relatives.

Family study data on normal controls are being analyzed. New family studies begun this year are childhood illness in offspring of patients versus controls, and anorexia nervosa as a possible genetic variant of affective disorders.

The completed and age collected prevalences in second degree relatives is even lower than our initial estimate. It appears that cultural factors reduce the rate of affective illness diagnosis in those generations by our methods, as noted above. The grandparents, uncles and aunts in this table include only those who had a self-report or a report from their own child, brother or sister.

Second Degree Relatives (Uncles, Aunts and Grandparents)

	Diagnosis of Relatives, %				
Diagnosis of Patient	S-A	BPI	BPII	UP	N
Schizoaffective (S-A)	0	0	0	2.2	49
Bipolar I (BPI)	0.6	1.1	0.4	5.4	577
Bipolar II (BP II)	0	0.5	0.5	3.6	210
Unipolar (UP)	0.7	0	0	3.7	144

2. Genetic Analyses of Affective Disorder in Families.

Single locus transmission of major affective disorder has been extensively analyzed in our sample of pedigrees using the final diagnoses of all individuals. The following samples of families were analyzed separately in attempts to minimize the clinical and biological heterogeneity of probands:

Type of Proband	Number of Families
BPI	75
BPII	22
UP	18
Schizoaffective	6
"low" MAO	20
Lithium Responders	18
"low" 5-HIAA	7

In addition, three analyses were performed separately in single large pedigrees.

Because of the uncertainty as to which diagnoses constitute the affective illness spectrum, we defined "illness" in the following ways:

1. BP, Schizoaffective, or UP with incapacitation
2. UP, BP, or Schizoaffective
3. (2) + suicide, acute functional psychosis and cyclothymic personality
4. (3) + alcoholism, drug abuse and sociopathy

The Elston-Stewart pedigree analysis method was used to test hypotheses of single autosomal and X-chromosome genetic transmission in addition to a hypothesis of non-genetic transmission. In most cases, all hypotheses were rejected. In some samples, the number of families was too small to allow for any discrimination among hypotheses. Thus, at least by the diagnosis criteria we have used and in the sub-groups we have defined so far, single genes do not account for the transmission of affective disorder. However, the possibility remains that we have not yet found the "right" subgroup of patients. In the next year, we will be examining the feasibility of more complicated models such as two-locus, polygenic, and mixed major gene and polygenic transmission of illness.

Since it is still possible that in some families, transmission is by a single gene, we tested for possible linkage of affective disorder with a series of genetic marker traits. Two loci possibly linked to illness are the MNS blood group and the Haptoglobin type (lod scores approximatley 1.5). In both cases, the likelihood of linkage is maximized using the first definition of illness. We are testing for linkage using both other criteria for illness and other subgroups of families to determine if there is any reasonable arrangement of the data favoring linkage. These lod scores are only mildly suggestive of linkage. However, if a linkage can be demonstrated in some subgroups of families, then this would indicate that at least in some cases there is single gene transmission of illness.

3. Linkage Studies of Enzymes of Catecholamine Metabolism (DBH and COMT).

We had previously demonstrated by pedigree analysis that the transmission of COMT and DBH activity in families was consistent with single, autosomal codominant genes. We have now tested for linkage of the genes for enzyme activity and approximately 20 genetic marker loci. A suggestive linkage has been found between the DBH gene and the ABO locus (the ABO locus is on chromosome 9.) The maximum lod score is 1.8 at recombination fractions of 0% and 10% in males and females, respectively. This result can be combined with that of a comparable study by Elston et al. to make a total lod score of 2.4 This score does not "prove" linkage but it is highly supportive. (Linkage is approximately 250 times more likely than non-linkage.) If this linkage is confirmed by other investigators, it would demonstrate concretely that a gene for DBH activity is located on chromosome 9.

4. Theoretical Studies in Genetics.

a) Simulation studies to test the power of pedigree analysis methods. We have extended our previous simulation studies (in collaboration with Dr. Kenneth K. Kidd at Yale U.) to determine how the addition of information on a genetic marker locus improves the power of the method to detect single gene

transmission of a "disease" trait. We find that in cases where a single locus is not detected by segregation analysis of the "illness", it can be detected with the additional information at the marker locus, but only if linkage is very tight (close to 0% recombination) and there are virtually no phenocopies.

b) Bias of linkage estimates due to population associations of diseases with genetic marker trait. If a disease is associated with a genetic marker trait in the population, estimates from linkage analysis may be biased by ignoring this association. We calculated the exact bias of the recombination fraction and the lod score in the presence of an association between a simple recessive disease and a two-allele codominant marker locus. The recombination fraction is slightly underestimated (not more than 5%) but the lod score can be inflated by as much as two-fold. These results are likely to hold for other single gene models of transmission as well. These biases are important when evaluating studies in the literature (especially those involving the HLA locus) where such population associations are present.

c) A critical review of adoption study methodology in psychiatry has been undertaken, with particular attention to unrecognized biases in some currently cited studies.

5. Cell Culture and Molecular Genetic Techniques.

Cell culture and molecular genetic techniques allow direct study of genetic variation between individuals. A cell culture laboratory for fibroblasts and lymphocyte/lymphoblast cultures has been set up this year, where the cells serve as possible models of cell membrane receptors and other processes in the nervous system. A cell lines collection was established with cells from a series of ten manic-depressive patients with one ill sibling of each patient, along with cells from series of normal controls including identical twins.

In fibroblasts, beta-adrenergic and muscarinic cholinergic ligands show specific binding activity and are now being characterized for comparability with CNS receptors.

In lymphocytes, the muscarinic cholinergic receptors reported in the literature were confirmed. Preliminary findings show increased ligand (QNB) binding in Bipolar manic-depressive patients when they are depressed but not in the well state. Corticosteroid receptors have been confirmed on the lymphocyte, and we are currently studying patients and controls.

Studies of ^{3}H-dihydromorphine binding to human granulocytes in our laboratory have indicated a specific high affinity binding site for beta-endorphin. This site is different pharmacologically from brain opiate receptors and appears to be similar to a beta-endorphin specific site previously reported on transformed lymphocytes. Efforts to replicate a previous report of a high affinity dopamine binding site on human lymphocytes were not successful. Although displacable ^{3}H-spiroperidol binding was demonstrated, it was not stereospecific in our studies.

A molecular genetics laboratory for study of genes for receptor proteins and CNS peptides, using recombinant DNA techniques, is now being equipped and established.

6. Biological and Genetic Clinical Studies

Genetic variation in neurotransmitter metabolism and receptors has been identified in twin studies. In collaboration with other laboratories, (Drs. Steven Paul and Wade Berrettini) platelet ^{3}H-imipramine binding, activities of serum glutamic acid decarboxylase (GAD) and platelet gamma-amino-butyric-acid-transaminase (GABA-T), and plasma GABA concentration have been identified as highly concordant in identical twins, and are being studied as possible genetic markers of affective disorder.

In collaboration with the laboratory of Dr. Marian Kafka we noted increased alpha-adrenergic receptor binding on platelets of affective illness patients as compared with controls.

7. Norepinephrine Metabolite Studies.

Using a newly developed, sensitive and specific gas chromatographic mass spectrometric assay of the norepinephrine metabolite methoxyhydroxyphenylglycol (MHPG), we have been pursuing a number of studies in healthy volunteers, depressed patients, and laboratory animals. In a study of healthy monozygotic twin volunteers, we showed high intraclass correlation for plasma free and total MHPG, suggesting heritability of metabolite levels. We have also shown that in healthy volunteers at rest, plasma MHPG levels are highly correlated with cerebrospinal fluid levels of the metabolite. The precursor metabolite DHPG, which exists in relatively high concentration in rat brain, was shown to be a minor metabolite in the human CSF samples.

Basal plasma MHPG levels appear to predict behavioral characteristics in healthy controls. We have shown that the pre-treatment metabolite concentration predicts excitation following amphetamine administration. In collaboration with Drs. Post, Ballenger and Uhde, we have found that higher plasma MHPG is related to lower MMPI scores for depression, hypochondriasis, and psychosthemia in control subjects; and that subjects with higher MHPG are less sensitive to painful stimuli than those with lower levels. In affectively ill patients, the plasma metabolite varies with shifts in psychotic state, being significantly higher in mania than depression. These state dependent changes appeared to be clearer than those noted in urinary metabolite excretion. In animal studies conducted in collaboration with Drs. Gilad, Fenerstein and Kopin, we have shown that measurement of plasma norepinephrine metabolites provides important information on noradrenergic function not apparent from levels of norepinephrine alone. Present work in laboratory studies is directed toward assay modifications allowing measurement of norepinephrine metabolites in small "punches" of specific brain nuclei.

Quantification of plasma metabolites provides a valuable index of drug effects in man. In collaboration with Drs. Pickar and Murphy, we have shown that decreases in plama MHPG during antidepressant treatment with monoamine oxidase inhibitors reflects relatively selective MAO-A inhibition. This would be the best available index of MAO-A inhibition in man, and may be a useful probe for assessing effective drug dose in individual patients. In collaboration with Drs. Uhde and Post, we have confirmed a previous report that clonidine treatment induces plasma MHPG in man. Preliminary results using this drug response as an index of presynaptic alpha-adrenergic receptor

sensitivity do not demonstrate differential responses between depressed patients and healthy controls. In data linking opiate effects with alterations of locus coeruleus activity, naloxone administration to healthy volunteers produced slight increase in plasma MHPG in a study conducted in collaboration with Drs. Naber and Pickar. In collaboration with Drs. Potter, Ross, Zavadil and Goodwin, we have shown that desipramine administration to healthy volunteers reduces deaminated plasma metabolites of norepinephrine, in agreement with a previous study of desipramine effects in laboratory rats.

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2. Gilad, G. and Jimerson, D.C.: Modes of adaptation of peripheral neuroendocrine mechanisms of the sympatho-adrenal system to short term stress as studied in two inbred rat strains. Brain Res. 206: 83-93, 1981.

3. Murphy, D.L., Pickar, D., Jimerson, D.C., Cohen, R.M., Garrick, N.A., Karoum, F. and Wyatt R.J.: Biochemical indices of the effects of selective MAO inhibitors (clorgyline, pargyline and deprenyl) in man. In Clinical Pharmacology in Psychiatry, Usdin, E., Dahl, S., Gram, L.F., and Lingjaerde, O. (Eds.). MacMillen Press, Ltd. In press.

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7. Pickar, D., Cohen, R.M., Jimerson, D.C., Lake, C.R. and Murphy, D.L.: Tyramine infusions and selective MAO inhibitor treatment. II. Interrelationships among pressor sensitivity changes, platelet MAO inhibition and plasma MHPG reduction. Psychopharmacology. In press.

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9. Post, R.M., Cutler, N.R., Jimerson, D.C. and Bunney, W.E., Jr.: Dopamine agonists in affective illness: Implications for underlying receptor mechanisms. In Proceedings of the International Symposium on Apomorphine and Related Dopamine Agonists. Sardinia, Italy, Sept. 29-Oct. 2, 1980. In press.

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Other Investigators Continued:

J.C. Gillin, M.D.	Chief, Unit on Sleep Studies	BP	NIMH
D.C. Jimerson, M.D.	Staff Psychiatrist	BP	NIMH
S.S. Jimerson, M.S.N.	Guest Worker	BP	NIMH
L. Kessler, M.D.	Guest Worker	BP	NIMH

Project Description:

Pharmacogenetic studies in psychopharmacology have largely been devoted to inherited differences in drug metabolism as opposed to genetics of physiologic and psychologic responses to drugs. The behavioral pharmacogenetics of psychoactive drugs in particular is an area in which little systematic investigation has been undertaken.

Dextroamphetamine (0.3 mgm/kg) or placebo was administered to 13 pairs of normal monozygotic twins, 3 pairs of dizygotic twins and 11 patients with bipolar affective illness in the well state and off medication. A behavioral excitation response to amphetamine was highly concordant in MZ twins, reproducible in an individual over time, and uncorrelated with plasma amphetamine level. An elation response was concordant and reproducible; it was correlated with amphetamine and is thus a pharmacokinetic effect of the drug. A depressive response was concordant but not reproducible.

Behavioral excitation in normal twins is predicted by the baseline variables of high plasma MHPG, low serum prolactin, and low pulse, and correlated with a rise in cortisol. Haloperidol pretreatment in one pair of twins abolished the excitation response but did not reduce rises in cortisol, growth hormone and prolactin. This suggests a role for dopamine in the excitation response but predominant serotonergic and noradrenergic mediation of the hormonal responses.

None of the responses or baseline measures distinguished patients from controls. Thus, consistently altered sensitivity to monoaminergic stimulation by amphetamine in Bipolar affective disorder was not demonstrated in this study. However, amphetamine-induced excitation was predicted by low plasma MHPG in patients and high MHPG in normal twins; this may reflect a different relationship between circulating catecholamines and behavior in affective illness.

In collaboration with Dr. Cohen and Dr. Pickar beta-endorphin levels were analyzed in patients and twins before and after dextroamphetamine. Endorphin levels rose significantly; the extent of the rise was correlated with the size of the cortisol response, demonstrating a possible coupling of release for these two peptide hormones.

Cholinergic agonists were also studied. Five patients and ten controls were studied in a pupillometry procedure which had demonstrated a patient control difference in miotic response to pilocarpine in a previous study. We did not replicate this finding and are examining further differences in the two studies (this might include time of year, hour of the day, method of drug administration.)

Eight Bipolar patients in the well state and off drugs, and 12 monozygotic twin controls have participated in a study of response to arecoline. Mood ratings are filled out before and after a dose of 8 mgm arecoline subcutaneously. Blood samples have been taken for cortisol, growth hormone, prolactin, and beta-endorphin. Preliminary analysis of mood rating does not show differences between patients and controls.

Proposed Course of Project.

Studies are under way to further specify the neurochemical basis of the heritable amphetamine responses found. These studies will employ haloperidol, propranolol and thymoxamine as blocking agents.

Pharmacogenetic studies of the serotonin and opiate systems are under consideration. We are also planning a nighttime study of cholinergic sensitivity, measuring hormonal responses and attempting to replicate our finding of a supersensitive cholinergic REM induction response in Bipolar patients.

Significance:

We have noted heritable variations in response to amphetamine in behavior, evoked responses, and hormone levels. These responses may be used as tools to examine neurochemical function in other patient or control groups. This is the first report of heritable variation in an affective response to stimulant drugs in man.

The excitation response to amphetamine is most probably dopaminergic while the hormonal responses are serotonergic and noradrenergic. Further neurochemical characterization of these responses will be significant for our understanding of inherited variation in normal brain function. We have no evidence, however, that the variations elicited by amphetamine play a role in the vulnerability to affective disorder.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00086-05 BP

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Outpatient Clinic for Genetic and Pharmacologic Studies of Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. S. Gershon, M.D.	Chief, Section on Psychogenetics	BP	NIMH
OTHER:	J. Nurnberger, M.D.	Senior Staff Fellow	BP	NIMH
	D. C. Jimerson, M.D.	Staff Psychiatrist	BP	NIMH
	S. Simmons, M.S.N.	Clinical Nurse Expert	CC	NURS
	M. S. Buchsbaum, M.D.	Chief, Section on Clinical Psychophysiology	BP	NIMH
	W. E. Bunney, Jr., M.D.	Chief	BP	NIMH
	W. Berrettini, M.D.	Staff Psychiatrist	BP	NIMH
	M. Kafka, Ph.D.	Research Associate	BP	NIMH
	A. Lewy, M.D.	Staff Psychiatrist	BP	NIMH
	D. Selinger, M.D.	Guest Worker	BP	NIMH
	L. Pons, M.D.	Guest Worker	BP	NIMH

COOPERATING UNITS (if any)
Clinical Center Nursing Department, NIH; Clinical Psychobiology Branch, NIMH; FDA

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Psychogenetics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.5	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The outpatient clinic provides a "well state" patient population for genetic and pharmacologic studies of persons with Bipolar (manic-depressive disorders and Unipolar (major depressive) disorders. Such a "well state" population is crucial in identifying biochemical, pharmacologic, or physiologic abnormalities which are present in persons vulnerable to affective disorder, and which may serve as genetic markers to affective illness. The clinic also provides a resource for studies of phenomenology of manic-depressive illness and treatment response to medications.

Studies of cholinergic sensitivity in Bipolar patients and relatives are continuing. We have tested mood and hormonal response to arecoline in euthymic patients and normal controls. This data is now being analyzed. We are also testing trihexyphenidyl, an anticholinergic agent, as an antidepressant in Bipolar patients. Four patients have been started in this protocol.

PHS-6040
(Rev. 10-76)

OTHER INVESTIGATORS CONTINUED:

L. Kessler, M.D. Guest Worker BP NIMH

Summary Continued:

Dr. Louis Pons has tested 45 clinic patients with a word association test and has demonstrated altered associative patterns in patients independent of state; the patterns tend to be normalized with increased lithium. In collaboration with Dr. Alfred Lewy we are testing sensitivity of patients to melatonin suppression by light; patients appear to be more sensitive than controls.

In collaboration with Dr. Marian Kafka, we have reported increased alpha-adrenergic receptor binding on platelets from bipolar patients in the "well state" and off medications.

Project Description:

The purpose of the project is to have a "well state" patient population available for participation in the following research areas:

1. Identification of biochemical, pharmacologic, or physiologic abnormalities which are present in persons who have had major affective disorder but who do not currently require hospitalization.

2. Identification of biological markers of a genetically transmitted predisposition to affective disorders.

3. Creation of a pool of available patients and relatives for further protocols which will be developed and submitted on the genetics of affective disorders, and on longitudinal studies of affective disorders.

Patients are admitted to the study upon referral from the wards of the NIMH intramural program or from various community referral resources. Seventy-five patients are currently enrolled in the BPB portion of the outpatient clinic.

Identification of biochemical, pharmacologic or physiologic vulnerabilities to affective illness: The strategy in this type of study is to identify a biologic vulnerability to illness which is not state dependent but present or evocable even when the patient is in the "well state" or in remission.

We have described the results of our twin study of amphetamine response (Z01 MH 00085-05 BP). Amphetamine response in patients has been studied using a similar paradigm. Responses in patients have been found not to differ from those in controls and thus the amphetamine challenge test is not likely to be useful as a trait marker of affective disorder.

Fifty-eight clinic patients participated in studies of lithium transport and $Na^+ - K^+$ ATPase (see Z01 MH 00081-07 BP).

Our studies of cholinergic responses in patients have also proceeded. The anticholinergic drug trihexyphenidyl (Artane) is being tested as an antidepressant. Five depressed patients in the clinic have been started on this protocol without difficulty. Blood samples before and after Artane have been taken by Dr. Suzan Nadi for cholinergic receptor binding studies. Several clinic patients and normal volunteer twins have also participated in a study of salivary response to arecoline by Dr. Drora Selinger.

Biochemical markers for affective illness have been sought in collaboration with other NIMH investigators. Dr. Marian Kafka has investigated alpha-adrenergic receptors on platelets in sixteen clinic patients and reports that the receptor binding is elevated in this group. Since these patients were euthymic and off medications, this may constitute a trait difference in affective illness. Dr. Wade Berrittini has tested levels of the putative neurotransmitter gamma-amino-butyric acid (GABA), and several associated enzymes in blood samples from clinic patients; results are now being analyzed. Ten clinic patients and their siblings had skin biopsies taken for cultured fibroblasts.

When depression in Bipolar patients is treated with conventional antidepressant drugs, episodes of mania and hypomania may be provoked and cycles of mania and depression may be induced. We are undertaking to study various antidepressant treatments in Bipolar patients looking for a treatment which will not induce mania but which is still an effective antidepressant. We are beginning with an anticholinergic agent (see above). Studies with zimelidine (a serotonergic agonist) and pirbuterol (a beta-adrenergic agonist) are now ready to begin.

Dr. Drora Selinger has studied lithium levels in the parotid gland secretions from eight patients. She reports a 0.97 correlation between parotid lithium level and serum lithium. This finding may be of great clinical importance for patients from whom it is difficult to obtain blood samples for lithium.

A word association test has been administered to 37 clinic patients by Dr. Louis Pons. He has found different patterns of response in our patient group than in controls tested with the same procedure here at NIMH. The patients show more originality of associations and less reproducibility, indicating a difference in cognitive processes even in this relatively euthymic group. There is a statistically significant association ($r = 0.5$, $P < .01$) between repetition of common responses and lithium level, implying normalization of association patterns with increased lithium.

We have collaborated with Dr. Alfred Lewy of the Clinical Psychobiology Branch in studying the melatonin response to light in affective illness. Normal human subjects will suppress melatonin production at night if they are in a room with lights considerably brighter than usual indoor lights. Patients, however, are more sensitive to light suppression. Eleven clinic patients have participated in this study so far. Five have been studied both on and off lithium (the others were studied in the lithium-free state.) We are currently considering genetic and pharmacologic studies to characterize this response more fully.

Significance of the Study:

Identification of a biologic vulnerability detectable in the well state in Bipolar manic-depressive illness would be of great importance for clinical treatment and also for identifying persons who might become ill in the future, including relatives of patients. Clinical and longitudinal studies of affective illness are important in increasing our knowledge of the course and treatment of this disorder.

Proposed Course of Project:

Studies of CNS and peripheral sensitivity to agonists and antagonists of monoamine and cholinergic receptors will continue. Other neurochemical systems (GABA, serotonin, endorphin) are also being tested. The in vitro studies, possibly using tissue culture and recombinant DNA techniques, now being developed in our laboratories should greatly expand the speed and convenience of our neurochemical markers studies.

The use of the clinic as a resource for the study of the clinical and biochemical characteristics of patients with affective disorder has been productive and will continue.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00117-06 BP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

α- and β-Adrenergic and Prostaglandin Receptors in Human Blood Elements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian S. Kafka	Physiologist	BP NIMH
	John F. Tallman	Chief, Section on Biochemistry and Pharmacology	BP NIMH
Other:	Craig C. Smith	Chemist	BP NIMH
	John W. Thomas	Chemist	BP NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Alpha- and β-adrenergic receptors and prostaglandin receptors are being studied in human blood cell preparations. The ability of adrenergic agonists to activate or inhibit adenylate cyclase is used as a measure of their biological function. Binding of tritiated adrenergic receptor antagonists to membrane receptors on the blood cells is being measured. These correlative measures are used to assess α- and β-adrenergic physiological function in normal human beings, patients with various psychiatric disorders, and patients receiving different psychopharmacologic agents.

PHS-6040
(Rev. 10-76)

Objectives:

(1) To develop methods to study the physiological function of α- and β-adrenergic receptors in easily obtainable human tissue.

(2) To study alterations in receptor number or function in pathological conditions characterized by altered receptor sensitivity and in psychiatric diseases.

(3) To provide an experimental model for the study of drug and/or physiologically induced changes in central receptor sensitivity in man.

(4) To understand the membrane perturbations or alterations connecting receptors occupied by their agonists with the inhibition or activation of adenylate cyclases and subsequent physiological events.

Methods Employed:

To measure α-adrenergic receptors, human platelets are prepared from freshly drawn blood. A portion of the platelets is washed and resuspended for measurement of cyclic AMP production. A second portion is homogenized to yield a membrane preparation which is washed and resuspended for the measurement of tritiated α-adrenergic antagonist binding. To measure β-adrenergic receptors a human leukocyte preparation is made from freshly drawn blood using a dextran polymer to induce rapid settling of the erythrocytes. The leukocyte-rich plasma is centrifuged at low speed to yield a leukocyte pellet. This pellet is washed several times in 0.9% saline solution and is used to measure binding of a tritiated β-antagonist and production of cAMP.

Cyclic nucleotides are determined by a competitive protein binding assay for cAMP or by radioimmunoassay for cGMP after incubation of intact leukocytes or platelets with the drugs under study. Adenylate cyclase activity is measured in platelet lysates.

Major Findings:

(1) Platelets from young male controls make less cyclic AMP than platelets from adult male controls.

(2) Platelets from schizophrenics make less cyclic AMP than platelets from control subjects.

(3) Platelets from patients with essential hypertension make less cyclic AMP than platelets from control subjects.

(4) The number of α-receptors is greater in platelets from schizophrenic than in platelets from control subjects.

Significance to Biomedical Research and the Program of the Institute:

The results obtained from these experiments are directly applicable to an assessment of receptor functioning in human disease states and in monitoring the

physiological effects of various drug treatments. If the peripheral α- and β-adrenergic receptors are related to or can serve as a model for central nervous system adrenergic receptors, valuable information may be obtained about the functioning of these central receptors in psychiatric diseases.

It is possible that adrenergic receptor function in the central and peripheral nervous systems is similar to that measured in platelets and leukocytes. Platelets from schizophrenic patients make less cyclic AMP. The decrease in cAMP production is due, at least in part, to the decrease in the activity of their platelet adenylate cyclase activity. If neurons in the central nervous system of schizophrenic patients also have decreased cAMP production, there may be a decrease in phosphorylation of, and activation of, some cell proteins, resulting in the deficits that characterize schizophrenia. If the number of α-adrenergic receptors is higher in the neurons, as well as in the platelets, of schizophrenic patients, transmission in the central nervous system may be altered: norepinephrine or epinephrine occupancy of increased numbers of pre- and post-synaptic α-adrenergic receptors might inhibit brain inhibitory centers, increasing excitation in parts of the brain under tonic inhibitory control. The excitation might contribute to the symptomatology and course of schizophrenia.

Proposed Course:

Examination of the mechanism of information transfer from α- and β-adrenergic receptors to adenylate cyclase is continuing. Studies of these receptors in patients with hypotension, affective illness, as well as further studies of α-receptor function in schizophrenia are underway.

Publications:

Gullner, H.G., Kafka, M.S., and Bartter, F.C.: Indomethacin increases leukocyte β-adrenoreceptors in man. Clin. Sci. 59: 397-400, 1980.

Kafka, M.S., van Kammen, D.P., and Kleinman, J.E.: The Alpha-adrenergic Receptor in Man: Normal Function and Pathology. In Usdin, E., Bunney, W.E. Jr., and Davis, J.M. (Eds.): Neuroreceptors--Basic and Clinical Aspects. New York, John Wiley, 1980, pp. 129-139.

Kafka, M.S., van Kammen, D.P., and Kleinman, J.E.: Alpha-adrenergic receptor function in schizophrenia. Psychopharmacol. Bull. 16: 91-94, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00119-05 BP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Putative Neurotransmitters and Modulators in Cerebrospinal Fluid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	Candace B. Pert	Pharmacologist	BP NIMH
	Craig C. Smith	Chemist	BP NIMH
	John W. Thomas	Chemist	BP NIMH
	David Pickar	Staff Psychiatrist	BP NIMH

COOPERATING UNITS (if any)

Unit on Drug Abuse, BP, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been incorporated into projects Z01 MH 00070-08 BP and Z01 MH00112-03 BP.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00124-04 BP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mechanism of Action of Lithium in the Treatment of Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Agu Pert	Psychologist	BP NIMH
Other:	Candace B. Pert	Pharmacologist	BP NIMH
	Jack E. Rosenblatt	Staff Psychiatrist	SMRC NIMH
	William E. Bunney, Jr.	Chief	BP NIMH
	Robert Hruska	Staff Fellow	OD NINCDS

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology, NIMH; Office of the Director, NINCDS

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Chronic treatment of rats with lithium for two and three weeks decreased [^{3}H]spiroperidol binding in the caudate nucleus by 26.3 and 26.9% respectively. One day after the termination of lithium treatment the binding was still 23.1% normal. Three days following termination of treatment it had recovered to normal levels. The in vitro addition of lithium to tissue homogenates from the striatum, cortex or hippocampus was found to inhibit the binding of [^{3}H]QNB. This effect was reversible since washing the tissue to remove the lithium removed the inhibition. Chronic exposure of rats to lithium did not produce any permanent alterations in the density of the binding sites or the affinity of the muscarinic cholinergic receptors.

PHS-6040
(Rev. 10-76)

It has recently been suggested that oscillations in catecholamine receptor sensitivity may underlie the etiology of affective disorders, especially manic-depressive illness. We have previously found that chronic lithium treatment appeared to prevent the development of dopamine receptor supersensitivity. These findings were taken to suggest that lithium may produce its therapeutic effects by dampening the oscillations in dopamine receptor sensitivity. Subsequent to these initial findings, we also demonstrated similar effects for the α- and β-adrenergic receptor systems. This series of studies was designed to further extend the analysis of the effects of lithium on neurotransmitter receptor sensitivity.

Effects of Chronic Lithium on Dopamine Receptors in the Caudate

Acute lithium has no effect on the binding of [^{3}H]spiroperidol when it is added to rat brain homogenates. We also failed to detect any effects of chronic lithium on the binding of [^{3}H]spiroperidol one week following the termination of chronic lithium treatment. In this study we examined the effects of chronic lithium on the binding of [^{3}H]spiroperidol in rat caudate following various intervals of chronic lithium treatment and also at various intervals after the termination of chronic lithium treatment.

Effect of Chronic Lithium on the Muscarinic Cholinergic Receptor

Behavioral data from our laboratory has suggested that there is an interactive effect between chronic lithium and cholinomimetic compounds. In this series of studies we evaluated the effects of chronic lithium on the binding of [^{3}H]QNB, a muscarinic cholinergic receptor ligand, in various regions of the brain following either chronic or acute (in vitro) lithium treatment. Rats were treated for three weeks with a lithium diet designed to induce lithium plasma levels of 0.7 - 0.9 mEq/liter. After three weeks the rats were sacrificed and the brains removed and frozen. The septum, hippocampus, cortex and striatum were dissected out and assayed for [^{3}H]QNB binding under various conditions. [^{3}H]QNB binding to homogenates from these brain regions was also evaluated following the addition of various concentrations of lithium to untreated rats.

Major Findings

Lithium added to caudate homogenates from rats fed a normal diet failed to have any effect on [^{3}H]spiroperidol binding in concentrations up to 30 mM. After one week of chronic lithium treatment, there was a small but non-significant reduction in [^{3}H]spiroperidol binding in the caudate nucleus. After two and three weeks, [^{3}H]spiroperidol binding had decreased to 26.3% and 26.9% of the control respectively. One day after the termination of chronic lithium treatment dopamine receptor binding was still significantly reduced. Three and ten days later the binding had returned to normal. These findings may explain the ability of lithium to block the increase in [^{3}H]spiroperidol binding following chronic haloperidol treatment. The latency of the lithium-induced reduction in [^{3}H]spiroperidol binding, along with the relatively rapid reversibility of the effect are consistent with the clinical characteristics of lithium's anti-manic effect.

The in vitro addition of lithium was found to inhibit the binding of [^{3}H]QNB to tissue homogenates prepared from the striatum, cortex or hippocampus. This in vitro inhibition of binding was reversible, since washing the tissue to remove the lithium removed the inhibition. Chronic exposure of rats to lithium in the feed did not produce any permanent alterations in the density of the binding sites or the affinity of the muscarinic cholinergic receptors. While chronic lithium does not alter receptor number, it does appear to interfere with muscarinic cholinergic receptors in vivo. While this phenomenon is temporary and recovers after removal of lithium, it may be important in states where the muscarinic cholinergic system is already partially compromised.

Significance to Biomedical Research and the Institute Program

Lithium is one of the most widely used and efficacious compounds in the treatment of mania and manic-depressive illness. Understanding its mechanism of action will aid in defining the factors underlying the expression of mania and manic-depressive illness.

Proposed Course

1. An effort will be made to evaluate the effects of lithium on the several populations of dopamine receptors.

2. A parametric analysis will be made of efficacy of chronic lithium to block dopamine receptor supersensitivity depending on the time of initiation of lithium and its termination in relationship to the chronic haloperidol treatment.

3. An effort will be made to evaluate the effects of lithium on several different receptor systems using autoradiographic techniques.

Publications

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00147-06 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Behavioral and Physiological Effects of Morphine-like Brain Peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Stress was found to decrease endorphins as well as bombesin in the periaqueductal gray matter (PAG) and a number of other brain regions regulating pain input. Substance P levels were not altered, however, by this manipulation. Microinjections of β-endorphin, morphine, neurotensin, VIP and bombesin into the PAG produced profound analgesia in the rat. Somatostatin, cholecystokinin, TRH, bradykinin, and substance P had no analgesic activity. Systemic injections of opiates were found to enhance dopaminergic activity using behavioral and biochemical procedures. Dopaminergic zona compacta neurons increased their basal firing rates following morphine while zona reticulata neurons decreased their firing rates. Opiate receptors have unique laminar distributions in the rat cerebral cortex.

PHS-6040
(Rev. 10-76)

Objectives

The objectives of this project are to define the mechanisms and sites of action of opiates and opioid peptides and to evaluate the effects of various environmental manipulations on the activity of central nervous system endorphin systems.

Effect of Stress on Brain Levels of Neuropeptides

Stress has been shown to induce potent analgesic effects (SA) in rats. It has been suggested by some that endorphins may play a role in mediating this phenomenon. The participation of endorphins in SA is supported by several recent findings. Peripherally, stress is known to release β-endorphin from the pituitary into the circulatory system. Thus, increased circulating levels of β-endorphin could be one factor involved in SA. Participation of endogenous opioids in SA is further supported by the ability of naloxone to partially antagonize analgesia induced by stressors. Stress analgesia has also been reported to alter brain levels of enkephalin and β-endorphin. In addition to endorphins, it is possible that a number of other neurohumors, especially neuropeptides, may be mobilized by stress to induce an analgesic state. We have recently reported that bombesin, a tetradecapeptide, is also a potent analgesic following injections into the periaqueductal gray matter (PAG), an area relatively rich in bombesin and bombesin receptors. Substance P, another neuropeptide with a high concentration in the PAG, also has been reported to produce analgesia following intracerebral as well as systemic injections. We have examined the effects of foot-shock stress in rats on levels of endorphins, substance P and bombesin in specific brain regions that have been implicated in pain processing. Following 10 minutes of foot-shock stress, the animals were decapitated and the brains rapidly removed and frozen. The basomedial hypothalamus, medial thalamus, PAG and the septum plus the bed nuclei of the stria terminalis were punched out from frozen coronal slices. Endorphin levels were assayed with the radioreceptor assay. Bombesin and substance P levels were assayed with the radioimmunoassay specific for these peptides.

Analgesic Effect of Opiate and Non-Opiate Neuropeptides

Injections of opiates into the PAG, which is high in opiate receptors and opiate peptides, produce profound analgesia in the rat. Besides endorphins, the PAG also contains relatively high concentrations of other neuropeptides. Since this region of the brain appears to be critical for pain perception, it was of interest to ascertain whether any other neuropeptides might also have analgesic properties following direct microinjections. Rats were prepared with chronic indwelling cannulae guides through which various neuropeptides could be injected into the PAG. Analgesia was assessed with both the tail-flick and hot-plate procedures.

Modulation of Nigrostriatal Dopamine Activity by Opiates

There is considerable evidence to suggest that opiates exert an important influence on the nigrostriatal dopamine system. The aim of this project was to localize and analyze the interactive effects between opiatergic and nigrostriatal dopaminergic neurotransmission using electrophysiological, biochemical and behavioral techniques.

Localization of Opiate Receptors in Brain

Although the rat cerebral cortex contains easily measurable quantities of opiate peptides, immunocytochemical procedures have revealed very few enkephalin-containing fibers in cortex. Since opiate receptors could be markers for "opiatergic" pathways in brain, we carried out a laminar analysis (translaminar autoradiographic grain counting) of different cortical areas which had been labeled by [^{3}H]naloxone or [^{3}H]D-Ala^2-D-Leu^5-enkephalin ([^{3}H]DADL), respectively, on slide-mounted rat brain sections which were then processed for autoradiography. In addition, we compared the distribution of binding sites in some areas to thalamocortical projections as determined by [^{3}H]amino acid transport.

Major Findings

Foot shock, sufficient in intensity to produce significant post-stress analgesia, was found to reduce endorphins in the PAG, medial thalamus and hypothalamus as assessed with the radioreceptor assay. No changes were found in the area which encompassed the septum and the bed nucleus of the stria terminalis (nst) or in the lumbar spinal cord. Concomitant with these decreases in brain levels, foot shock also increased the endorphin content of cerebrospinal fluid. Bombesin-like immunoreactivity was also reduced in the PAG, hypothalamus and septum + nst but not in the spinal cord or medial thalamus. Substance P-like immunoreactivity, on the other hand, was not altered by foot shock in any of the above brain regions or spinal cord. Since injections of both bombesin and endorphins into the PAG have been reported to produce analgesia, it is proposed that part of the analgesic effects of SA is mediated, at least in part, by the prolonged activation of endorphinergic and bombesinergic circuitry in the PAG resulting in the consequent depletion of these peptides in this brain area. Substance P, on the other hand, is probably not involved in SA since levels of this peptide were not altered by foot shock in any brain area or the spinal cord.

Of the opiates, β-endorphin exhibited the most potent analgesic effects in both the hot-plate and tail-flick tests. Both procedures revealed it to be approximately ten times as potent as either morphine or DADL. D-Ala^2-met-enkephalin, on the other hand, appeared to be approximately one-third as potent as either morphine or DADL.

Bombesin, VIP and neurotensin all exhibited analgesic effects. Bombesin was the most potent non-opiate peptide analgetic in the hot-plate test, while VIP was the most potent in the tail-flick test. In general, all of these peptides were approximately three times more potent than morphine following PAG injections.

β-endorphin-induced analgesia was readily antagonized by naloxone. Analgesia induced by either bombesin, neurotensin or VIP, on the other hand, was not reversed by naloxone. Injections of β-endorphin and morphine into the mesencephalic reticular formation (MRF) produced considerably less analgesia than direct injections into the PAG. Injections of bombesin, VIP and neurotensin into the MRF also increased hot-plate latencies but had no effect on the tail-flick response. Injections of TRH, somatostatin, cholecystokinin 26-33 (non-sulfate) and bradykinin had no appreciable analgesic activity following PAG injections. Contrary to some reports, substance P was not found to have any substantial

analgesic properties from a dosage range of 0.001-27 μg. Only the highest doses produced a very transitory (5 minutes post-injection) elevation of hot-plate and tail-flick latencies.

It is clear that there is considerable redundancy in the neurochemical coding of the endogenous pain suppression mechanisms at the level of the PAG and MRF. In our studies rats lesioned unilaterally in the substantia nigra (SN) with 6-OHDA rotated ipsilateral to the lesion following systemic injections of morphine. We have found this rotational behavior to increase in intensity with repetitive administration. Direct unilateral microinjections of opiates into the SN induce rotational behavior contralateral to the injection. This rotational behavior can be blocked by injections of haloperidol into the ipsilateral caudate nucleus. Unilateral injections of opiates into the SN also induce a significant elevation in homovanillic acid in the ipsilateral caudate.

Single-unit recording techniques were also used to study the effects of systemically administered morphine on the activity of rat SN dopamine (SN-DA) neurons and a specific subpopulation of SN zona reticulata (SN-ZR) neurons. It has been reported that i.v. morphine increases the firing rate of SN-DA neurons but decreases the firing rate of unidentified SN-ZR neurons. Our results confirm and extend these findings. SN-DA neurons increase their firing rate an average of 70% above baseline following administration of 4.0 mg/kg i.v. morphine. This increase is completely reversed by 0.01 mg/kg of i.v. naloxone. The class of SN-ZR neurons we examined have an inhibitory influence on SN-DA neurons and were identified using previously established criteria. These SN-ZR neurons were more sensitive to morphine than SN-DA neurons and decreased their firing rate an average of 26% below baseline following 1.0 mg/kg i.v. morphine. Naloxone, 0.01 mg/kg i.v., led to a consistent rebound increase in firing rate of these SN-ZR neurons to 29% above baseline. No such rebound was seen in SN-DA neurons following naloxone.

The laminar distribution of grains differs markedly across areas with the pattern depending upon which radioligand is used as a receptor label. We also observed correlations between the laminar distribution of "μ"-like binding sites and thalamocortical projections to different regions. For example, the distribution of both [^{3}H]naloxone sites in frontal cortex and [^{3}H]amino acids transported from the mediodorsal nucleus shows a sharp decline in density from the most superficial part of layer III. [^{3}H]DADL sites, in contrast, show a shallower layer I peak and a broad peak extending from layers III to VI. The medial frontal cingulate and retrosplenial areas all show layer VI peaks of [^{3}H]naloxone binding; however, the layer III binding peak disappears in posterior retrosplenial cortex. The insular and entorhinal areas are densely labeled in layer VI by [^{3}H]naloxone; piriform cortex is sparsely labeled throughout. Insular and piriform cortex are the only limbic cortical areas to show more overall [^{3}H]DADL than [^{3}H]naloxone binding. Unique to primary sensory cortices is [^{3}H]naloxone labeling in upper layer V. In addition, area 17 can be easily discriminated from surrounding area 18 by a lower density of labeling in layer VI. Because of evidence that apparent multiple opiate receptors may be functionally different conformations of the same receptor, we suggest that laminar differences in [^{3}H]naloxone and [^{3}H]DALA binding reflect an *in vitro* stabilization of different receptor conformations which undergo dynamic interconversions *in vivo*.

Significance to Biomedical Research and the Institute Program

Since opiates are among the most potent psychomimetic and euphorogenic compounds, it is of special significance that there exist endogenous compounds in brain which are apparently mimicked by opiates. There is substantial evidence that these compounds may be novel neurotransmitters or neuroregulators. If this is the case, then it is quite conceivable that such neurohumors may play an important role in affective states. Furthermore, dysfunctions in the neuronal systems encoded by these peptides may underlie some of the etiology of mental disorders and neurological dysfunctions.

Proposed Course

1. Electrophysiological and behavioral techniques will be used to analyze the functions of cortical opiate and non-opiate peptides in the primate.

2. The effects of opiate and non-opiate neuropeptides on basal activity of nigral neurons will be investigated using single-cell recording and pressure-ejection techniques.

3. Further attempts will be made to define environmental and physiological conditions that can modify enkephalin and other peptide levels in brain.

4. Attempts will be made to define the precise relationship of cortical opiate receptors to known cortical projections.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00157-03 BP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies in Benzodiazepine Binding Sites in the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	0.7	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on the mechanism of action of the benzodiazepines indicate that benzodiazepines alter neuronal inhibition in the central nervous system through a GABA receptor/benzodiazepine/ionophore complex. Additionally, there seems to be a second type of benzodiazepine binding site, characteristic of non-neuronal cells, in various preparations including kidney and glial cells. The control of synthesis and structure of these benzodiazepine receptors has been carried out and attempts to purify these receptors are underway.

PHS-6040
(Rev. 10-76)

Many of the pharmacological actions of the benzodiazepines can be attributed to their actions on γ-aminobutyric acid (GABA) systems in the brain. Electrophysiological studies on dorsal raphe neurons indicate that the benzodiazepines act postsynaptically to potentiate GABAergic inhibition in this midbrain nucleus. Direct binding studies have shown that both in vitro and in vivo binding of [^{3}H]diazepam to a specific high-affinity benzodiazepine binding site in cerebral cortical tissue are enhanced by the direct in vitro addition of GABA and GABA agonists or by pretreatment of animals with GABA analogs and agents that elevate GABA levels in brain. Ontogenic development of [^{3}H]diazepam binding in brain parallels the development of the sodium-independent [^{3}H]GABA binding. The ability of GABA to enhance benzodiazepine binding is present throughout development and inversely related to age. These data suggest that there is a functionally significant interaction between the benzodiazepines and GABA throughout development and at maturity. The model, proposed to relate these interactions, is that there exists a benzodiazepine/GABA/Cl ionophore complex.

Two types of benzodiazepine binding sites for [^{3}H]diazepam in mammalian central nervous tissue can be identified using selective in vitro tissue culture and in situ kainic acid lesion techniques. These two binding sites were pharmacologically distinguished by differential displacement of the [^{3}H]diazepam radioligand using the centrally active benzodiazepine, clonazepam, and the centrally inactive benzodiazepine, RO5-4864. Clonazepam-displaceable binding sites were found to be located principally on neuronal membranes, while RO5-4864-displaceable binding sites were found to be located on non-neuronal elements including kidney. These pharmacological distinctions can be used to characterize the predominant cell types which bind benzodiazepines in nervous tissue. It is suggested that one quantitative measure of different cell populations is the ratio of clonazepam- to RO5-4864-displaceable [^{3}H]diazepam binding within a single neuronal tissue sample.

Both the brain-specific and peripheral-type benzodiazepine receptors have been solubilized using various detergents. The properties of these sites are being clarified. In addition, the specific photoaffinity labeling of membrane-bound and detergent-solubilized benzodiazepine binding sites has been investigated using UV irradiated [^{3}H]flunitrazepam as a photochemical probe. The time course and the regional and pharmacological specificity of the photolabeling reaction has been determined for "brain-specific" benzodiazepine binding sites; "peripheral-type" binding sites treated in an identical manner were not specifically labeled. Comparison of the number of sites labeled and blocked by [^{3}H]flunitrazepam photolabeling of detergent-solubilized preparations indicated that about one site was blocked and unavailable for reversible binding for each site photolabeled. In contrast, when membrane-bound sites were photolabeled, about four sites were inactivated for each site photolabeled. Examination of photolabeled binding sites from various brain regions including cortex, striatum, and hippocampus using SDS-PAGE gave only a single labeled band of apparent molecular weight 48,000.

Significance to Biomedical Research and Institute Program:

These studies have been directed towards a more complete understanding of the pharmacology and biochemistry of the GABA receptor/benzodiazepine/ionophore complex. Based on electrophysiological and receptor binding studies, it is clear

that the benzodiazepines alter neuronal inhibition in the central nervous system. The mechanism for this enhanced inhibition is obviously complex, but studies on membrane environment and on the biochemical characterization of the isolated binding site will lead to a more comprehensive understanding of the mode of action of benzodiazepines and, perhaps, brain mechanisms in general. The benzodiazepines are among the most widely prescribed drugs in the world.

Proposed Course:

Continued studies on the function of the benzodiazepine binding site in neuronal inhibition are expected. We also hope to further characterize the isolated binding site and membrane environment of this receptor.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00159-02 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neurotransmitter Receptors in the Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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1.6	1.6	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Neurotransmitter receptors are being studied in the rat central nervous system. Brain neurotransmitter receptors undergo circadian rhythms which may be altered by changed physiological function, the seasons of the year, and drugs.

PHS-6040
(Rev. 10-76)

Objectives:

(1) To develop methods to measure the presence of neurotransmitter receptors in the nervous system.

(2) To measure whether alterations in receptor number accompany alterations in function in the central nervous system.

(3) To measure rhythms in brain receptors and study their alterations with changes in function and with drugs.

RAT SUPERIOR GANGLION

Methods Employed:

The specific binding of tritiated α-adrenergic antagonist to membranes from the rat superior cervical ganglion is used to measure the number of α-adrenergic receptors. In some experiments ganglia are decentralized (pre-ganglionic fibers from the central nervous system severed) a week before the experiment, removed the day of the experiment and compared with intact ganglia freshly removed from the contralateral side of the rat. In other experiments, ganglia are removed and cultured, and compared with ganglia freshly removed from the rat. Electrical measurements were made post-synaptically with a single electrode and pre-synaptically with suction electrodes.

Major Findings:

(1) Membranes from the rat superior cervical ganglion contain α-adrenergic receptors. [^{3}H]Dihydroergocryptine, an α-adrenergic antagonist, binds specifically, saturably and reversibly to the receptor. Specific binding is defined as that part of total binding displaced by a saturating concentration of phentolamine.

(2) α-Adrenergic agonists l-epinephrine and l-norephinephrine inhibit binding potently, whereas the β-adrenergic agonist l-isoproterenol is weak. α-Adrenergic antagonists phentolamine, dihydroergocryptine, and piperoxane are potent inhibitors of binding, and the β-antagonist dl-propranolol, a weak inhibitor. Binding of [^{3}H]dihydroergocryptine, then, is to an α-, not a β-, adrenergic receptor.

(3) Decentralization decreases the number of α-adrenergic receptors by 50% without a change in K_D, suggesting that the number, rather than the affinity of α-adrenergic receptors, is decreased by decentralization.

(4) Agonist occupancy of α-receptors inhibits Ca^{++}-influx into the pre- and post-ganglionic neurons and inhibits neurotransmission through the ganglion.

Significance to Biomedical Research and the Program of the Institute:

Membranes in the rat superior cervical ganglia contain α-adrenergic receptors. When the axons of cholinergic pre-ganglionic fibers to the ganglion degenerate, 50% of the α-receptors are lost, suggesting that one-half of the α-receptors are

present on pre-ganglionic endings located presynaptically with respect to the adrenergic neurons of the ganglion. Electrophysiological experiments show that alpha occupancy of both pre- and post-ganglionic α-receptors inhibits the transmission of a single impulse across the ganglion and modulates the transmission of a train of impulses.

α-Receptors on ganglia are being measured during alterations in the ganglion function in organ culture.

RHYTHMS IN BRAIN NEUROTRANSMITTER RECEPTORS

Methods Employed:

The specific binding of tritiated ligands to membranes prepared from rat brains is used to measure rhythmic changes in receptor binding in rats sacrificed at intervals over a 24-hour period, over the course of the year, with and without physiological alterations and drug treatment. The α-adrenergic receptor is measured with the specific binding of [^{3}H]WB4101; the β-adrenergic receptor, with [^{3}H]dihydroalprenolol; the dopamine receptor, with [^{3}H]spiroperidol; the muscarinic acetylcholine receptor, with [^{3}H]QNB; the opiate receptor, with [^{3}H]naloxone; and the benzodiazepine receptor, with [^{3}H]diazepam.

Major Findings:

(1) There are circadian rhythms in the number of α- and β-adrenergic, dopamine, opiate, and muscarinic acetylcholine receptors.

(2) The rhythms of all of these are altered by chronic treatment with the antidepressant drug, imipramine.

Significance to Biomedical Research and the Program of the Institute:

These experiments document the existence of circadian rhythms in some brain neurotransmitter receptors. The rhythms are in the number of receptors, not the affinity of each receptor for its tritiated ligand. As transmission across CNS synapses is modulated by receptors, the change in the number of a given receptor, both pre- and post-ganglionically, as well as the relationship between that receptor and other receptors, and the relationship of all to the light-dark cycle could have a profound effect on neuronal activity patterns in the brain. In addition, the alteration of receptor rhythms by chronically administered imipramine may help to elucidate the therapeutic role of imipramine in alleviating depression in patients.

Proposed Course:

The rhythms in α- and β-adrenergic, dopamine, opiate, acetylcholine and other receptors are being measured with additional drugs and after functional alterations. The relationship of the rhythms to the "biological clock" is being examined.

Publications:

Kafka, M.S., Horn, J.P., Thoa, N.B., and McAfee, D.A.: α-Adrenergic receptors in the rat superior cervical ganglion. Bulletin of the New York Academy of Sciences, 358: 342-345, 1980.

Naber, D., Wirz-Justice, A., and Kafka, M.S.: Circadian rhythms of catecholamine receptor binding in the rat brain and their modification by chronic imipramine. Drug Research 30: 1235-1236, 1980.

Naber, D., Wirz-Justice, A., Kafka, M.S., and Wehr, T.A.: Dopamine receptor binding in rat striatum: ultradian rhythm and its modification by chronic imipramine. Psychopharmacology 68: 1-5, 1980.

Wirz-Justice, A., Kafka, M.S., Naber, D., and Wehr, T.A.: Chronic imipramine shifts α- and β-adrenergic receptor circadian rhythms in rat brain. Life Sci. 27: 341-347, 1980.

Wirz-Justice, A., Wehr, T.A., Goodwin, F.K., Kafka, M.S., Naber, D., Marangos, P., and Cameron, I.: Antidepressant drugs slow circadian rhythms in behavior and brain neurotransmitter receptors. Psychopharmacol. Bull. 16: 46-47, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00160-02 BP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Control of β-Adrenergic Receptors in Cultured Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John F. Tallman	Chief, Section on Biochemistry and Pharmacology	BP NIMH
Other:	Richard C. Henneberry	Senior Scientist	LMB NINCDS
	Peter H. Fishman	Research Biochemist	DMN NINCDS
	Craig C. Smith	Chemist	BP NIMH
	Pierre Mallorga	Visiting Fellow	BP NIMH

COOPERATING UNITS (if any)

Laboratory of Molecular Biology, NINCDS
Laboratory of Clinical Science, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

β-Adrenergic receptors are present in a number of cultured cell lines and their ability to function is altered by a number of factors in the culture medium and the length of time in culture. When astrocytoma cells (C6 glioma) undergo many generations, the ability of the cell to respond to catecholamines is profoundly reduced. This reduction in responsiveness is due to several factors including a decreased number of β-adrenergic receptors and increased phosphodiesterase activity; this results in a 5-fold decrease in cAMP half-life in the cell. Ultimately, these findings may be important in understanding age-related phenomena. On the other hand, avian muscle cells (myoblasts) held in culture will fuse to form myotubes which respond to catecholamines. The process of fusion is correlated with the appearance of new synthesis of β-adrenergic receptors which appear to couple with a pre-existent adenylate cyclase.

PHS-6040
(Rev. 10-76)

Responsiveness to catecholamines was studied in two different strains of rat glioma C6 cells. The C6 cells of low passage possessed a high capacity to accumulate cyclic AMP in response to (-)-isoproterenol. Cholera toxin was also able to stimulate cyclic AMP accumulation in these cells. High passage C6 cells were unresponsive to (-)-isoproterenol or to cholera toxin, except in the presence of a high concentration of phosphodiesterase inhibitor. The affinity of β-adrenergic receptors on both strains for (-)-[^{3}H]dihydroalprenolol was similar; however, C6 low passage possessed several times the number of β-adrenergic receptors found in C6 high passage. This difference correlated with the difference found in (-)-isoproterenol-stimulated adenylate cyclase between C6 low passage and C6 high passage cells. The sodium fluoride-stimulated adenylate cyclase was similar in both strains. Cyclic AMP phosphodiesterase activity was similar in both strains. Cyclic AMP phosphodiesterase activity was 2- to 3-times higher in homogenates of C6 high passage cells than in C6 low passage cells. In intact cells, the rate of breakdown of cyclic AMP was 5-times faster in C6 high passage than in C6 low passage cells. Thus, differences in β-adrenergic receptor number and phosphodiesterase activity explain, in part, the lack of responsiveness of C6 high passage cells. Our studies indicate that continuous subculturing of rat glioma C6 cells led to complex alterations in the β-adrenergic receptor-adenylate cyclase system.

Catecholamine-induced desensitization was compared in the same strains of rat glioma C6 cells. When exposed to isoproterenol, C6 low passage cells accumulate high levels of cyclic AMP (3-4 nmol/mg protein), whereas C6 high passage cells do not. After prolonged exposure to isoproterenol, both C6 low passage and C6 high passage cells exhibited a diminished response when rechallenged with the agonist. The desensitization process was both time and dose dependent and similar parameters were observed for C6 low passage and C6 high passage cells. C6 high passage cells exposed to isoproterenol in the presence of a phosphodiesterase inhibitor initially accumulated large amounts of cyclic AMP but the inhibitor did not alter the time course of agonist-induced desensitization. In addition, the inhibitor, which by itself elevated cyclic AMP levels more than did isoproterenol by itself in C6 high passage cells, did not induce a refractory state.

Agonist-induced loss in β-adrenergic receptors was much slower than the rate of desensitization. Membranes from desensitized cells, however, exhibited an apparent lower affinity for agonist as measured by agonist displacement of labeled antagonist binding. A substantial loss of isoproterenol-stimulated adenylate cyclase activity in membranes prepared from agonist-treated C6 low passage and C6 high passage cells was observed without a significant loss in sodium fluoride-stimulated activity.

Isoproterenol-treated C6 high passage cells remained completely responsive to cholera toxin. Desensitized C6 low passage cells exhibited a reduced response to the toxin which was partially overcome by the phosphodiesterase inhibitor. Activation of cyclase, however, was not reduced in either C6 low passage or C6 high passage cells treated with agonist and then toxin. Furthermore, cyclase was activated to the same extent when membranes from control or desensitized cells were incubated with the A_1 subunit of cholera toxin and nicotinamide adenine dinucleotide. We conclude that the large accumulation of cyclic AMP in C6 low passage cells exposed to β-agonists does not mediate the subsequent desensitization process. Instead, the initial stages of catecholamine-induced desensitization in both C6 low passage and C6 high passage cells appear to represent a

specific uncoupling of the β-adrenergic receptor from a functional regulatory component of adenylate cyclase.

The appearance of β-adrenergic receptors and isoproterenol-stimulated adenylate cyclase during differentiation of embryonic quail muscle cells in tissue culture has been examined. β-Adrenergic receptors first appear during the fusion stage of myogenesis. [^{125}I]Iodohydroxybenzylpindolol ([^{125}I]IHYP) was used as the ligand to characterize β-adrenergic receptors. In equilibrium binding studies, mature myotubes contained, per diploid nucleus, approximately 800 specific and saturable binding sites, which have a dissociation constant (K_d) for [^{125}I]IHYP of 108 pM. When determined from the association and dissociation rate constants, the kinetically derived K_d is 63 pM. [^{125}I]IHYP binding to myotube membranes is blocked stereoselectively by a number of β-adrenergic agonists and antagonists. In contrast, proliferating myoblasts contain fewer than 10 β-adrenergic receptors per cell.

Both myoblast and myotube membranes contain an adenylate cyclase activity which is stimulated by guanine nucleotides and fluoride, and is activated in membranes derived from cells treated with cholera toxin. Myotube membrane adenylate cyclase, but not myoblast membrane adenylate cyclase, is stimulated 2 to 3 fold by isoproterenol. Accumulation of cyclic AMP by intact myotubes, but not by myoblasts, is stimulated 3 to 4 fold by isoproterenol. Both cell types accumulate cyclic AMP in response to cholera toxin. These results indicated that as the embryonic muscle cells fuse, they develop specific β-adrenergic receptors that become functionally coupled to a pre-existing adenylate cyclase.

Significance to Biomedical Research and the Program of the Institute:

The advantages of a model system to study receptor dynamics and function are tremendous compared to studies with animals. As cells in culture which are readily manipulated provide a stable source of cells which respond in a consistent fashion, the effects of drugs at known concentrations at the cell surface may be studied. The examination of drug effects in the model system can lead to predictions of *in vivo* effects of these drugs in patients. It is also possible to study subsensitivity in this model system which may be related to clinical subsensitive states.

Proposed Course:

Research is currently underway into the following questions:

1. What is the mechanism of catecholamine-induced subsensitivity?

2. What is the role of membrane fluidity in desensitization?

Publications:

Fishman, P.H., Mallorga, P., and Tallman, J.F.: Catecholamine-induced desensitization of adenylate cyclase in rat glioma C6 cells: evidence for a specific uncoupling of β-adrenergic receptors from a functional regulatory component of adenylate cyclase. Mol. Pharmacol. in press, 1981.

Mallorga, P., Tallman, J.F., Hirata, F., Henneberry, R.C., and Axelrod, J.: Mepacrine blocks β-adrenergic agonist-induced desensitization in astrocytoma cells. Proc. Nat. Acad. Sci. USA 77: 1341-1345, 1980.

Mallorga, P., Tallman, J.F., and Fishman, P.H.: Differences in the β-adrenergic responsiveness between high and low passage rat glioma C6 cells. Biochim. Biophys. Acta, in press, 1981.

Parent, J.B., Tallman, J.F., Henneberry, R.C., and Fishman, P.H.: Appearance of β-adrenergic receptors and catecholamine responsive adenylate cyclase activity during fusion of avian embryonic muscle cells. J. Biol. Chem. 255: 7782-7786, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00166-02 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Peptide Secretory Products of Oat Cell Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Candace B. Pert	Pharmacologist	BP NIMH
Other:	Desmond Carney	Clinical Associate	MO NCI
	John D. Minna	Chief	MO NCI

COOPERATING UNITS (if any)

Medical Oncology Branch, NCI

LAB/BRANCH
Biological Psychiatry Branch

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Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Work on this project has been temporarily delayed.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00167-02 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neurochemical Coding of Brain Pathways Revealed by Autoradiography

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Candace B. Pert	Pharmacologist	BP NIMH
Other:	Miles A. Herkenham	Psychologist	LNP NIMH
	Wayne D. Bowen	Staff Fellow	BP NIMH
	Rémi Quirion	Guest Worker	BP NIMH

COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NIMH;

LAB/BRANCH
Biological Psychiatry Branch

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Section on Biochemistry and Pharmacology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.2	0.3

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We continue to study the distribution of brain neurotransmitter or drug receptors by our newly developed autoradiographic method. Quantitation of receptor distribution with tritium-sensitive film is now also possible. Specifically, chemically coded neuronal pathways can be surmised from the concordance of receptor distributions and autoradiographic track tracing techniques. Furthermore, adjacent sections can be incubated under different conditions with different ligands to reveal information about receptor subtypes.

PHS-6040
(Rev. 10-76)

Objective:

To map the neuroanatomical distribution of various chemically coded pathways in brain.

Methods Employed:

1. Newly developed in vitro autoradiography--Unfixed frozen brain tissue is melted onto slides, incubated in appropriate radioactive ligands to label receptors, washed thoroughly, dried rapidly, fixed with paraformaldehyde vapors and dipped in radio-sensitive liquid emulsion for traditional autoradiographic workup.

2. Receptor-labeled slides are placed tightly against tritium-sensitive film for two weeks and then grain density is transformed by computer.

3. Old-fashioned "grind and bind", whereby tissue dissection is carried out followed by homogenization and study of specific binding to homogenates after rapid filtration.

Major Findings:

1. Opiate receptors can be visualized as early as prenatal day 15 in developing rat brain. Surprisingly, opiate receptors appear early, even before cells have migrated into final position, and there is some indication that the opiate receptor cell surface plays a role in directing ultimate position in brain.

2. Autoradiographic evidence has revealed that claims about multiple opiate receptor subtypes (μ, δ, κ, etc.) are inaccurate. Rather, the opiate receptor appears quite flexible--it assumes different conformational states characterized by different ligand selectivity patterns depending upon whether it is coupled to adenylate cyclase (δ), inactive (κ), or desensitized (μ).

3. There are, indeed, phencyclidine (angel dust) receptors with a very well-defined structure activity relationship which correlates with behavioral data. These receptors are congregated in cortical areas and have a marked affinity for the psychotomimetic (σ) opiates as well as ketamine and other angel dust analogs.

4. There is a marked and striking gradient of conformationally complex Type I opiate receptors which corresponds to sensory processing hierarchies in the rhesus monkey cortex.

5. The striking patchy pattern of Type I opiate receptors in rat striatum enjoys a perfectly fitted mosaic reciprocal relationship with projections from the parafascicular nucleus of the thalamus.

Significance to Biomedical Research and Program of the Institute:

Pinpointing neurochemically coded tracts by this defined neuroanatomical procedure will enable us to perform lesioning and drug mimicry experiments to determine the functional significance of each newly discovered pathway. The method can be used on human brain and ultimately should give information about the contribution of various neurochemically coded tracts to pathology.

Proposed Course:

We plan to follow up suggestions that the more complex opiate receptor is located in the most evolutionary new advanced areas of brain, as well as being concentrated in the phylogenetically newest animals (e.g., human). We also plan to study the distribution of other receptors to reconstruct the neuronal circuitry in a similar manner.

Publications:

Bowen, W.D. Gentleman, S., Herkenham, M., and Pert, C.B.: Interconverting μ- and δ- forms of Type I opiate receptors in rat striatal patches. Proc. Nat. Acad. Sci. USA, in press.

Herkenham, M. and Pert, C.B.: In vitro autoradiography of opiate receptors in rat brain suggests loci of "opiate-ergic" pathway. Proc. Nat. Acad. Sci. USA 77: 5532-5536, 1980.

Herkenham, M. and Pert, C.B.: Mosaic distribution of opiate receptors parafascicular projections and acetylcholinesterase in the rat striatum. Nature 291: 415-418, 1981.

Kent, J.L., Pert, C.B., and Herkenham, M.: Autoradiographic visualization of opiate receptor ontogeny in rat forebrain. Develop. Brain Res., in press.

Lewis, M.E., Mishkin, M., Bragin, E., Brown, R.M., Pert, C.B., and Pert, A.: Opiate receptor gradients in monkey cerebral cortex: correspondence with sensory processing hierarchies. Science 211: 1166-1169, 1981.

Moody, T.M., Taylor, D.P., and Pert, C.B.: Effects of guanine nucleotides on CNS neuropeptide receptors. J. Supramol. Struct., in press.

Pert, A., Moody, T.W., Pert, C.B., DeWald, L.A., and Rivier, J.: Bombesin: receptor distribution in brain and effects on nociception and locomotor activity. Brain Res. 193: 209-220, 1980.

Pert, C.B.: Type I and Type II opiate receptor distribution in rat brain--what does it tell us? Adv. Biochem. Psychopharmacol. 28: 117-131, 1981.

Pert, C.B. and Herkenham, M.: From Receptors to Brain Circuitry. In Cagan, R. (Ed.): Proceedings of the International Symposium on the Biochemistry of Taste and Olfaction. New York, Academic Press, in press.

Quirion, R., Hammer, R.P. Jr., Herkenham, M., and Pert, C.B.: The phencyclidine (angel dust)/σ "opiate" receptor: its visualization by tritium-sensitive film. Proc. Nat. Acad. Sci. USA, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00168-02 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Physiological Function of Neurotransmitter Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Candace B. Pert	Pharmacologist	BP NIMH
Other:	Susan Gentleman	Pharmacologist	BP NIMH

COOPERATING UNITS (if any)

Diabetes Research Laboratory, Veterans' Administration Hospital

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been incorporated into project Z01 MH 00169-01 BP.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00169-01 BP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Allosteric Receptor Modulation and Altered Sensitivity States

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Candace B. Pert	Pharmacologist	BP NIMH
Other:	Uwe Kurt Schumacher	Chemist	BP NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Neurotransmitter receptors appear to be capable of profound conformational mobility, as they couple to effectors in the cell membrane. Often, the writhings of the flexible receptor protein molecule result in alterations in ionic flux. The conformational state of receptors differ in altered states of super- and subsensitivity. For example, the excessive endorphin levels circulating in the obese mouse provides an interesting model of a subsensitive opiate receptor.

PHS-6040
(Rev. 10-76)

Objectives:

To understand the relationship between obesity, insulin secretion, peripheral endorphin levels and opiate receptors. To understand the nature of the benzodiazepine receptor's regulation of the chloride ionophore.

Methods Employed:

Radioreceptor assay and radioimmunoassay of opiate peptides. Radioreceptor assay for benzodiazepines.

Major Findings:

1. Chronic administration of the opiate antagonist naltrexone slows the weight gain of obese mice while restoring elevated insulin levels toward normal and restoring elevated pituitary endorphin levels toward normal as well. Opiate receptors in the pancreas which regulate insulin secretion appear responsible for the effect.

2. The disulfonic stilebene blockers of ion channels have selected effects on GABA and benzodiazepine receptor binding, thus, adding more evidence to the notion that the chloride ionophore is intimately associated with these two receptors.

3. Supersensitive dopamine receptors can be demonstrated in the kidney of spontaneously hypertensive rats.

4. Massive endorphin release, and perhaps opiate receptor desensitization, occurs following transauricular electroacupuncture.

5. An aspect of lithium's mode of action is to stabilize dopamine receptor sensitivity oscillations, a finding which continues to be supported by the data accumulated this year.

Significance to Biomedical Research and Program of the Institute:

The notion that alterations in mood are a function of oscillations in neurotransmitter receptor sensitivity is perhaps the most exciting new lead in attempting to understand the causes of mental illness. Studies aimed at elucidating the molecular mechanism of receptor super- and subsensitivity are thus extremely relevant to understanding psychiatric disease.

Proposed Course:

We plan future experiments to explore the relationship between the conformational state of the opiate receptor as deduced by its ligand selectivity pattern and the state of its receptor sensitivity.

Publications:

Bunney, W.E. Jr., Pert, A., Rosenblatt, J.E., and Pert, C.B.: Some Biological Considerations of the Mode of Action of Lithium in the Treatment of Affective Illness. In Kline, N.S., Gershon, S. and Schou, M. (Eds.): Proceedings of the International Lithium Conference: Controversies and Unresolved Issues. Amsterdam, Excerpta Medica, in press.

Costa, T., Russell, L., Pert, C.B., and Rodbard, D.: Halide and GABA-induced enhancement of diazepam receptors in rat brain: reversal by disulfonic stilebene blockers of anion channels. Mol. Pharmacol. in press.

DiMicco, J.A., Kopin, I.J., and Pert, C.B.: Evaluation of β-Adrenergic Receptor Status by Plasma Sampling in Living Rats. In Yamamura, H.I., Olsen, R.W. and Usdin, E. (Eds.): Psychopharmacology and Biochemistry of Neurotransmitter Receptors. Amsterdam, Elsevier/North Holland, 1980, pp. 237-246.

Naber, D., Cohen, R.M., Pickar, D., Kalin, N.H., Davis, G., Pert, C.B., and Bunney, W.E. Jr.: Episodic secretion of opioid peptides in human plasma and monkey CSF: evidence for a diurnal rhythm. Life Sci. 28: 933-935, 1981.

Pert, A., Dionne, R., Ng, L., Bragin, E., Moody, T.W., and Pert, C.B.: Alterations in rat central nervous system endorphins following transauricular electroacupuncture. Brain Res. in press.

Pert, A., Pert, C.B., Davis, G., and Bunney, W.E. Jr.: Opiate Peptides and Brain Function. In van Praag, H.M. (Ed.): Handbook of Biological Psychiatry, Vol. II, Part IV. New York, Marcel Dekker, in press.

Pickar, D., Cutler, N.R., Naber, D., Post, R.M., Pert, C.B., and Bunney, W.E. Jr.: Plasma opioid activity in manic-depressive illness. Lancet, April 26, 1980, p. 937.

Recant, L., Voyles, N.R., Luciano, M., and Pert, C.B.: Naltrexone reduces weight gain, alters "β-endorphin", and reduces insulin output from pancreatic islets of genetically obese mice. Peptides 1: 309-313, 1981.

Rosenblatt, J.E., Shore, D., Wyatt, R.J., and Pert, C.: Increased renal [^{3}H]spiroperidol binding in spontaneously hypertensive rats. Eur. J. Pharmacol. 67: 317-320, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00326-08 CN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Neuropharmacology and Psychobiology of Mania and Depression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dennis L. Murphy	Chief	CN NIMH
OTHER:	Robert M. Cohen	Staff Physician	CN NIMH
	Thomas Insel	Staff Physician	CN NIMH
	Ned H. Kalin	Staff Physician	CN NIMH
	Samuel C. Risch	Staff Physician	CN NIMH
	Larry J. Siever	Staff Physician	CN NIMH
	Benjamin Roy	Staff Physician	CN NIMH
	Carol F. Hoover	Social Worker	CN NIMH
COLLAB:	Iain C. Campbell	Visiting Scientist	CN NIMH
	Edward F. Donnelly	Psychologist	SMR NIMH
	Stanley L. Slater	Staff Physician	DIR NICHD

COOPERATING UNITS (if any)

Nursing Department, Unit 6D
Clinical Center Rehabilitation

LAB/BRANCH
Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.2	3.2	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In investigations bearing on the status of brain neurotransmitter systems in patients with affective disorders, new evidence suggesting decreased α-adrenergic receptor responsiveness in some patients with depression has been obtained. Several intensive case investigations were made of patients who may represent prototypes of a depressive subgroup with increased noradrenergic availability and decreased noradrenergic receptor responsivity. In studies of the cholinergic neurotransmitter system, depressive-type symptoms were identified following physostigmine administration in normals. The development of these symptoms correlated with β-endorphin, cortisol and prolactin changes, perhaps representing a state of hypersensitivity in the hypothalamic-pituitary axis in susceptible individuals.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Individuals with depression, mania, and related disorders with affective components, including individuals with schizoaffective and characterologic disorders, are studied in attempts to understand the psychological and biological mechanisms involved in therapeutic drug effects in these disorders. As individual differences in psychoactive drug responsiveness appear to depend upon many psychological and biological factors, a variety of study approaches are utilized.

Methods Employed:

1. Behavioral and psychological assessment: Pretreatment evaluation of patients requires information from interviews of the patient and family, from psychometric approaches, from direct behavioral observation using various quantitative scales and from patients' self-ratings. The elucidation of individual and patient subgroup differences in drug response depends upon this information obtained by the clinical staff, including psychiatrists, psychologists, social workers and nursing personnel. Subsequent evaluation of drug response depends upon objective behavioral assessment as well as self-rated psychological change as obtained from a number of quantitative scales, several of which have been developed on this unit.

2. Biological assessment: Plasma, platelets, urine, and cerebrospinal fluid are collected for the measurement of enzymes, levels of biogenic amines and their metabolites, other chemical variables, and drug levels. Electrophysiologic measurement of sleep and of cortical evoked potentials are accomplished in collaborative studies with investigators in the Biological Psychiatry Branch.

Major Findings:

The Branch's current studies with psychoactive drugs fall into three main areas: (1) investigations of the status of the noradrenergic neurotransmitter system in depression and in relation to antidepressant drug administration; (2) investigations of the central cholinergic neurotransmitter system in depressed patients and in normals; and (3) continuing studies with monoamine oxidase-inhibiting antidepressants. In addition, other studies have evaluated smooth pursuit eye movements in depressed patients and normals; the occurrence of thought disorder in patients with primary affective disorders; and self-reported mood changes and other behavioral side effects of lithium administration to depressed patients.

Dr. Larry Siever has initiated a series of investigations in conjunction with the Biological Psychiatry Branch and the Clinical Psychobiology Branch to ascertain the role of noradrenergic receptor alterations in the affective disorders and their modification by antidepressant (particularly clorgyline) treatment. One study (performed in collaboration with Dr. Thomas Uhde and Dr. Robert Post) has been using clonidine, a selective α_2-adrenergic agonist, as a pharmacologic challenge of the noradrenergic system in affectively

disordered and obsessive-compulsive patients prior to and during antidepressant treatment. Clonidine stimulates growth hormone by acting on postsynaptic α_2-adrenergic hypothalamic receptors; the plasma growth hormone increase produced by clonidine is blunted in most depressed patients, suggesting decreased responsiveness of these central α_2-adrenergic receptors. Furthermore, the magnitude of the growth hormone response has been found to correspond inversely to pretreatment plasma free MHPG concentrations in these patients, suggesting that increased noradrenergic release may be associated with decreased α_2-adrenergic responsiveness, a finding predicted from basic principles of receptor adaptation. The growth hormone response to clonidine in rapidly cycling bipolar patients is also being examined in collaboration with Drs. Richard Hauger and William Potter. Preliminary data from these studies led to a case report concerning two patients with very aberrant noradrenergic indices including extreme elevations in plasma norepinephrine; both had reduced blood pressure responses to tyramine and blunted growth hormone responses to clonidine. These may represent prototypes of a depressive subgroup with increased noradrenergic availability and decreased noradrenergic receptor responsivity centrally and peripherally.

In other ongoing work, Dr. Siever and coworkers have also evaluated the blood pressure decrease and MHPG decrease in response to clonidine as potential indices of presynaptic α_2-adrenergic autoreceptor responsivity. The blood pressure decrease appears to be blocked during chronic but not acute antidepressant treatment, suggesting that autoreceptors may subsensitize with chronic treatment. This would have the effect of lessening the feedback inhibitory impact of the increased norepinephrine availability induced by antidepressants. We are also evaluating platelet α_2-adrenergic receptor status in collaboration with Dr. Marian Kafka. We have observed increases in α_2-adrenergic receptor binding in unipolar depressed patients. We are now examining the relationships between α_2-adrenergic receptors and plasma norepinephrine levels during clorgyline treatment with Dr. C. R. Lake.

A specific, testable hypothesis integrating animal studies conducted by Dr. Robert Cohen and other clinical evidence suggesting that supersensitive α_2-adrenoreceptors play an important role in the etiology and maintenance of affective illness has been reviewed in recent publications first authored by Dr. Robert M. Cohen and by Dr. Siever. The proposals are based on the following observations: (1) changes in adrenergic receptors in the periphery and brains of rats in response to antidepressant regimens; (2) new studies of the monoamine oxidase type A-inhibiting antidepressant clorgyline, specifically relating to adaptation in the α-adrenergic presynaptic negative feedback system; (3) human peripheral α-adrenergic receptor changes from studies of patients with affective illness; and (4) observations from animals and humans experiencing stress and withdrawal from chronic amphetamine and opiate administration, suggesting that the development of supersensitive α-adrenoreceptors may lead to affective illness in vulnerable individuals.

In studies of the cholinergic neurotransmitter system, Dr. Risch and coworkers have extended their earlier studies of the behavioral and neuroendocrine responses to single doses of physostigmine and arecholine (two cholinergic agonists) in depressed patients to studies in non-psychiatric

patient populations. Normal volunteers, screened for the absence of a personal or family history of affective disorders and free of concurrent marijuana usage, received intravenous infusions of high dose physostigmine or saline in a randomized, double-blind, counterbalanced paradigm. Self-rating and observer ratings both demonstrated a statistically significant, physostigmine associated increase in depressive-type symptoms in the group as a whole, particularly pronounced in certain individuals. These results are the first report of physostigmine associated depressive symptomatology in normal subjects. While these findings are different from two previous studies, our use of drug-sensitive self-rating scales, and some differences in physostigmine dosage and infusion times may have contributed to our results. These findings suggest that high dose physostigmine may represent a pharmacological model of depression in normal subjects, or alternatively may be diagnostic of vulnerability to affective disorder in certain subjects free of a previous history of affective disturbances. In addition, on the basis of correlations between mood responses and β-endorphin changes in the studies in patients, Dr. Risch has developed evidence suggesting that a cholinergically mediated adrenocorticotropic (ACTH)/β-endorphin pathway may modulate mood and cognition in man, and that affective disorder patients and normal subjects sensitive to the dysphoric effects of centrally active cholinomimetics may exhibit hypersensitivity of this hypothalamic pituitary axis.

Publications:

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Insel, T.R., van Kammen, D.P., Cohen, R.M., Alterman, I.S., and Murphy, D.L.: Lithium and mood change in affective disorder patients. Biol. Psychiatry, in press.

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Siever, L.J., Pickar, D., Lake, C.R., Kopin, I.J., Cohen, R.M., and Murphy, D.L.: Extreme elevations in plasma norepinephrine associated with decreased α-adrenergic responsivity in two depressed females. Am. J. Psychiatry, in press.

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Siever, L.J., Insel, T., and Uhde, T.: Noradrenergic challenges in the affective disorders. J. Clin. Psychopharmacol., in press.

Murphy, D.L., Roy, B., Pickar, D., Lipper, S., Cohen, R.M., Jimerson, D., Lake, C.R., Muscettola, G., Saavedra, J., and Kopin, I.J.: Cardiovascular changes accompanying mnoamine oxidase inhibition in man. In Usdin, E., Weiner, N., and Creveling C. (Eds.): Function and Regulation of Monoamine Enzymes: Basic and Clinical Aspects. Hampshire, England, MacMillan, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00327-09 CN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Dominance in the Marriages of Affective Disorder Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. F. Hoover, D.S.W. Social Worker CN, NIMH

COOPERATING UNITS (if any)

Department of Psychiatry, Thomas Jefferson University, Philadelphia, Pa. (R. G. Fitzgerald, M.D.)

LAB/BRANCH
Clinical Neuropharmacology Branch

SECTION
NIMH:ADAMHA:NIH:CNB

INSTITUTE AND LOCATION
NIMH:ADAMHA:NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study of dominance in marriage compares patients with primary affective disorders, their spouses, and individuals from the community.

PHS-6040
(Rev. 10-76)

Objectives:

Project Description:

42 patients and their spouses, as well as 30 "normal" pairs from the community were administered the Conflict in Marriage Scale (CIMS), an agree-disagree card sort. A discriminant function analysis was performed to establish significant differences between patients, spouses, and community individuals. Outstanding among these differences were responses on family decision making, including variations between husbands and wives in the affective group. These results were further interpreted in relation to clinical material from interviews.

Major Findings:

Patients with affective disorders were less likely to make final decisions on family matters (17%) than their spouses were (64%). 78% of women patients, compared to 50% of community women, believed their husbands made final decisions. A mere 3% of community husbands thought their wives made such decisions, compared to 47% of men patients who attributed these decisions to their wives.

Varying perceptions on dominance by husbands and wives in these marriages leads to speculation concerning possible assortative mating as well as cumulative interaction effects leading to a characteristic dominance tilt between partners

Publications:

Hoover, C.F., and Fitzgerald, R.G.: Marital conflict of manic-depressive patients. Arch. Gen. Psychiatry 38:1:65-67, 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00328-06 CN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Monoamine Oxidase Activity and Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dennis L. Murphy	Chief	CN NIMH
OTHER:	Larry J. Siever	Staff Physician	CN NIMH
	Robert M. Cohen	Staff Physician	CN NIMH
	Monte S. Buchsbaum	Section Chief	BP NIMH
	Toomas Eisler	Visiting Scientist	ET NINCDS
	Pauline Lerner	Staff Fellow	BP NIMH
	E. S. Gershon	Section Chief	BP NIMH
	A. J. Sostek	Staff Fellow	DSMR NIMH
	A. P. Zis	Staff Fellow	CP NIH

COOPERATING UNITS (if any)

Department of Psychology, Univ. of Maryland (Dr. Robert D. Coursey)
Department of Psychiatry, Yale University (Dr. Donald E. Redmond, Jr.)

LAB/BRANCH
Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	2.0	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In studies evaluating possible correlations between platelet monoamine oxidase (MAO) activity and behavior, differences in arousal responses and motor activity patterns were found when human newborns with high versus low amine oxidase activities were compared. These characteristics are possibly analogous to behavioral and psychological features which have recently been more explicitly delineated as associated with low MAO activity in normal populations and certain psychiatric patient populations. In laboratory studies of monoamine oxidase, dopamine has been found to be predominantly deaminated by the MAO B subtype in non-human primates and man.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project is investigating the apparent relationships between monoamine oxidase (MAO) activity and behavior. Because individual differences in MAO activity are most readily studied in man using blood platelets as sources of the enzyme, the project has also had as an objective the study of factors which affect platelet MAO activity and which may be relevant to the interpretations of differences in MAO activity found in psychiatric patient subgroups. The study of the mechanisms of action of drugs which inhibit MAO activity, and the consequences of MAO inhibition on amine neurotransmitter metabolism and other aspects of brain function are additional objectives.

Methods Employed:

Tissues obtained for the study of monoamine oxidase characteristics include human and other mammalian platelets, brain, heart, liver and kidney. Dr. Redmond has provided platelets and other tissues from vervet and rhesus monkeys as well as several other non-human primate species. Drs. Coursey, Buchsbaum, Sostek and Zis have obtained samples from normals. Several different MAO assay procedures have been adapted or developed in this laboratory for the study of enzyme activity in platelets and other tissues.

Major Findings:

A series of studies have shown that monoamine oxidase (MAO) activity in human platelets is essentially identical in properties to MAO type B measured in other human tissues including brain. According to twin and family investigations in normals, platelet MAO activity is predominantly under genetic control. Differences in MAO activity between various psychiatric patient groups and controls have been frequently reported, with alcoholic and bipolar I affective disorder patients most often found to have reduced platelet MAO activity, while in some studies patients with secondary or non-endogenous unipolar affective disorders have been found to have higher MAO activity. Chronic schizophrenic patients also have reduced MAO activity, although neuroleptic treatment may contributed to the observed platelet MAO changes in this population. Family members of alcoholic and bipolar affective disorder patients also have MAO values and/or incidences of these disorders different from controls, a finding which has been interpreted as suggesting that altered MAO activity may not be just a marker for these disorders, but might represent a familial or even genetic vulnerability factor or risk factor. However, inconsistencies across the different reports in the literature and various ways in which exogenous factors (including non-specific stress factors and drug treatment) may have affected the results limit the conclusions which can be drawn from many of the present investigations in patients.

Our current studies have investigated human newborns using objective, interactive behavioral assessment techniques and umbilical cord platelet and

plasma amine oxidase activity determinations to evaluate possible relationships between these measures prior to significant postnatal experience. Infants with lower platelet monoamine oxidase (MAO) and/or lower plasma amine oxidase activity were more highly aroused, more motorically active and more difficult to console than those with higher MAO activity. These behavioral characteristics are remarkably similar to the more elaborate affective and social features which have previously been associated with MAO activity differences in adult humans and rhesus monkeys.

As part of a continuing series of studies to identify factors capable of influencing MAO activity, intravenous dopamine infusions were found to elevate plasma and platelet amine oxidase activities in normals and patients.

In other laboratory studies, dopamine was found to be predominantly deaminated by the MAO-B subtype in non-human primate and human brain. This B form of the enzyme is also the only MAO present in human platelets.

In recently-completed analyses of biochemical changes resulting from the administration of selective MAO inhibitors to man, consistent reductions in platelet MAO-B activity and marked, non-overlapping elevations in urinary phenylethylamine excretion in subjects treated with pargyline and deprenyl provided clear evidence that MAO-B activity was markedly inhibited during treatment with these two MAO-B inhibitors in all patients. These two measurements would thus seem to provide the most useful indices of MAO-B inhibition during chronic treatment with MAOI's in man. Reduction in plasma and CSF MHPG concentrations were maximal with clorgyline treatment, and may provide the most useful index of MAO-A inhibition, in the absence of a readily available human tissue source of the enzyme, as is available for MAO-B activity assay in human platelets. The combined data indicate that in the doses used, clorgyline maintained high selectivity as an MAO-A inhibitor, and deprenyl as an MAO-B inhibitor, while pargyline more markedly inhibited MAO-B, but also inhibited MAO-A. Selectivity was maintained with clorgyline and deprenyl for at least a 4-week period of drug administration in man.

Publications:

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Zuckerman, M., Buchsbaum, M.S., and Murphy, D.L.: Sensation seeking and biological correlates. Psychological Bull. 88:187-214, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00329-06 CN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Platelets as models for the study of neurotransmitter function.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jonathan L. Costa	Staff Physician, CNB	NIMH
COLLAB:	E. D. Eanes	Chief, LBS	NIDR
	E. Weinbach	Chief, LPD	NIAID
	K. L. Kirk	Chemist, LC	NIAMDD
	L. Powers	Member Tech. Staff, Bell Lab, Murray Hill, N.J.	
	B. Chance	Professor Johnson Res. Fdn., Philadelphia, Pa.	
	R. Blumenthal	Chief, LMB	NCI
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COOPERATING UNITS (if any)
Stanford Synchroton Radiation Laboratory, Stanford, CA; Bell Laboratories, Murray Hill, NJ; Johnson Research Foundation, Philadelphia, PA

LAB/BRANCH
Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION
NIMH:ADAMHA:Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The processes of amine uptake and amine storage have been explored using human platelets, isolated storage vesicles, chromaffin vesicles, and model systems. The actions of psychoactive drugs known to affect amine disposition have been examined using mitochondria isolated from rat brain and liver. Techniques used include measurement of fluxes of radiolabelled tracers, enzymatic and biochemical assays, atomic-absorption spectroscopy, polarographic recording of oxygen uptake, and use of synchroton radiation to study extended atomic fine structure and near edge fine structure. The studies extend our understanding of the mechanisms responsible for amine movement across membranes, the actions of psychoactive drugs, and the chemical and physical processes responsible for amine storage in vesicles.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Work on this project continues the evaluation of the biochemical basis for the function of psychoactive drugs, the biophysical and biochemical factors responsible for amine uptake and storage in vesicles, and the relationship between the structural arrangement of vesicle components and their amine storage capacity.

Studies Implemented: (1) Isolation of storage vesicles (dense bodies) from human platelets, quantitation of yields, and measurement of the uptake of labeled amines by the isolated vesicles; (2) evaluation of the uptake of fluorinated analogues of serotonin; (3) examination of the effects of psychoactive drugs (e.g., reserpine, pargyline) and neurotransmitter amines on oxidative phosphylation and proton fluxes in mitochondria isolated from rat brain and liver; (4) formation of synthetic precipitates with a composition identical to the core of human-platelet storage vesicles ("synthetic dense bodies"); (5) measurement of the binding of amines to synthetic dense bodies and analogues of chromaffin vesicles; (6) examination of the extended atomic fine structure and near edge fine structure of calcium in synthetic dense bodies and isolated chromaffin vesicles.

Methods Employed:

1. General Preparative Procedures. Platelets were isolated from normal volunteers by differential centrifugation or by plateletphoresis. Bovine adrenal glands were collected at the slaughterhouse. Platelet storage vesicles and chromaffin vesicles were prepared by homogenization and differential centrifugation with metrizamide or sucrose gradients. Mitochondria and mitoplasts were prepared from rat liver or brain by homogenization and differential centrifugation.

2. Examination of Amine Fluxes in Intact Platelets and Isolated Vesicles. Radiolabelled amines or fluorinated amine analogues were used to measure uptake rates in intact platelets and isolated storage vesicles. The amount of amine taken up per vesicle was estimated by counting vesicles in whole mounts with the electron microscope. The electron microprobe attachment was used to facilitate accurate identification of individual vesicles.

3. Examination of Mitochondrial Responses to Psychoactive Drugs. The oxygen consumption of isolated mitochondria or mitoplasts was measured polarographically with an oxygen electrode. Mitochondria in various states of respiration were perturbed by the addition of reserpine, pargyline, or biogenic amines. Changes in enzyme activity were measured spectrophotometrically or by fluorescence spectroscopy.

4. Preparation and Analysis of Models for Amine Storage Vesicles. Utilizing an apparatus designed to form precipitates under carefully controlled conditions, precipitates were prepared with a composition essentially identical to the core of storage vesicles of human platelets. The synthetic precipitates were then used to explore factors influencing core

formation, changes in the core during the platelet secretory process, and the binding of biogenic amines by the core material. As analogues of chromaffin vesicles, large unilamellar vesicles containing 0.2 M ATP were prepared by sonication and reverse-phase evaporation of dioleyl-phosphatidylcholine suspensions. Uptake of the amines quinacrine and 9-aminoacridine was followed by fluorescence spectroscopy.

5. X-ray Absorption Studies of Calcium in Storage Vesicles and Model Systems. Platelets, isolated dense bodies, and chromaffin vesicles were prepared for study by rapid freezing in ethylene glycol or dimethyl-sulfoxide. Synthetic analogues of dense bodies and model compounds were prepared by filtration of solids onto polycarbonate filters. The x-ray absorption spectrum of the calcium in each system was recorded and processed by computer to extract information about the geometry around the average calcium atom.

Major Findings:

1. Uptake of Serotonin by Isolated Storage Vesicles: With appropriate preparative techniques, from 10-20% of the total number of dense bodies in a platelet preparation can be separated and recovered. The maximal rate of serotonin uptake, expressed per dense body, is only about 10% of that observed in intact platelets. Thus, as hypothesized from previous studies, under normal conditions uptake into vesicles may require juxtaposition between plasma and vesicle membranes.

2. Uptake of Fluorinated Serotonin Derivatives: Two ring-fluorinated serotonin derivatives, 6-fluoro-serotonin and 4,6-difluoro-serotonin, have 5-hydroxyl groups with pKa's respectively one and two orders of magnitude below that for native serotonin. Nevertheless, uptake into the non-releasable and vesicular compartments proceeds at a similar rate for all three compounds, suggesting that the zwitterion is not the transported form. Since the 4,6-compound cannot move from the non-releasable into the vesicular pool, however, the electronic configuration around the 5-hydroxyl group appears to have critical requirements for this step.

3. Mitochondrial Responses to Psychoactive Drugs: Reserpine at micromolar levels acts as a classical uncoupling agent for mitochondria isolated from liver and brain when added with all three of the most commonly-used substrates. However, this action is observed only if the reserpine is dissolved in organic solvents prior to addition to the mitochondrial suspension, and thus appears to require penetration through the mitochondrial outer membrane for action. This conclusion is supported by the observation that reserpine in water uncouples respiration in isolated mitoplasts (mitochondria free of outer membranes). Pargyline and the biogenic amines benzylamine and phenethylamine, in contrast, do not affect any of the four states of mitochondrial respiration. Mitochondria from rats injected chronically with pharmacologic doses of pargyline or reserpine show no respiratory alterations.

4. Preparation and Characterization of Models for Human-Platelet Dense Bodies: Synthetic precipitates with a composition identical to that of platelet dense bodies can be formed reproducibly and in large quantities.

Their exact composition appears to be determined in part by the composition of the initial mixture and in part by specific binding constraints in the solid. The synthetic precipitates are in equilibrium with the supernatant solution, and change composition or dissolve extremely rapidly when diluted with sodium chloride solutions (i.e., under conditions analogous to the secretion of platelet dense bodies). Although the solids evolve protons during formation, and on a weight basis are associated with fewer protons than the equivalent amount of nucleotides and pynphosphate in solution, they contain sufficient protons to make the interior of a

5. Amine Binding to Analogues of Storage Vesicles. The binding of aqueous solutions of quinacrine, 9-aminoacridine, or serotonin to synthetic analogues of chromaffin vesicles or platelet dense bodies is of low affinity, non-saturable at the concentrations known to load storage vesicles in vivo, and not of sufficient magnitude to account for observed amine storage capacity. Consideration of mechanisms for amine storage other than direct binding of amines to constituents of the vesicle thus seems warranted.

6. Synchroton Studies of Calcium in Storage Vesicles. The extended atomic fine structure of the calcium in dense bodies of intact human platelets and isolated dense bodies is similar and typical of amorphous systems such as the synthetic analogues of dense bodies. Further analysis of this data and the near edge fine structure should provide a more quantitative understanding of the structure of the dense body.

Significance to Biomedical Research and the Program of the Institute. These studies continue the exploration of the factors governing amine uptake and storage in human platelets. The work has also been extended to the chromaffin vesicle, and has employed isolated mitochondria to examine the actions of various psychoactive drugs and biogenic amines. The extent of participation of the vesicle membrane and core in amine uptake and storage has been defined in more rigorous terms, and the molecular achitecture of the components is better understood. Insights gained in these studies should be applicable to other aminergic systems, and may provide a basis for understanding alterations in amine storage under certain pathological conditions.

Publications:

Eanes, E.D., Costa, J.L., MacKenzie, A. and Warburton, W.K.: Technique for the preparation of solid specimens for X-ray absorption studies. Rev. Sci. Instruments. 51:1579-1580, 1980.

Costa, J.L., Kirk, K.L. and Stark, H.: Anomalous compartmentation of 5-hydroxytryptamine in human platelets. Br. J. Pharmacol. 72:449-459, 1981.

Costa, J.L., Murphy, D.L. and Stark, H.: Applicability of models for carrier-mediated serotonin transport in human platelets. J. Physiol. (in press).

Costa, J.L.: Internalization of serotonin as a consequence of its association with human platelets at 0°C. Biochem. Pharm. (in press).

Eanes, E.D., Powers, L., and Costa, J.L.: Extended x-ray absorption fine structure (EXAFS) studies on calcium in crystalline and amorphous solids of biological interest. Cell Calcium. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00330-03 CN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Use of Electron and Photon Imaging Techniques to Study Aminergic Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	J. J. Bailey	LAS	DCRT
COLLAB:	J. P. Wall) --	Electron Microscopists,	
	J. A. Hainfield) ---	Brookhaven National Lab.	
	P. Chaudhari	Chief, Physical Sciences, IBM Research Labs.	
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COOPERATING UNITS (if any)
Brookhaven National Laboratories, Upton, L.I., N.Y.; IBM Thomas J. Watson Research Laboratory, Yorktown Hts., N.Y.; Maxwell Laboratories, San Diego, Ca., State Univ. of New York at Stonybrook, Stonybrook,, L.I., N. Y.

LAB/BRANCH
Clinical Neuropharmacology Branch

SECTION
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INSTITUTE AND LOCATION
NIMH:ADAMHA:Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Electrons and long-wavelength x-rays have been used to continue the study of intact human platelets and platelet dense bodies in hydrated and dehydrated specimens. Techniques employed include electron energy-loss spectroscopy, electron microprobe analysis, mass-thickness measurements, contact x-ray microscopy, and scanning x-ray microscopy. Computer image processing methods have been developed to analyze the data.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To develop techniques which combine imaging of features on a nanometer scale with determination of the elemental and chemical composition of subcellular organelles. The techniques are used to examine the structure and dynamics of various organelles which store amines.

Studies Implemented: (1) Electron microprobe analysis of model systems and the calcium and phosphorus content of dense bodies in intact platelets and following biochemical isolation; (2) development of quantitation routines for measuring the nitrogen, phosphorus, calcium, and oxygen content of model systems and platelet dense bodies utilizing electron energy-loss spectroscopy; (3) examination of platelets and model systems by scanning x-ray microscopy; (4) mass thickness measurements of model systems, isolated dense bodies, and dense bodies in intact platelets after various treatments known to alter their composition; (5) computer processing of mass-thickness data; (6) examination of hydrated platelets by flash x-ray microscopy; (7) development of new techniques for x-ray contact microscopy, and application to studies of intact platelets.

Methods Employed:

1. General Preparative Procedures. Air-dried platelet whole mounts of fixed or unfixed platelets were prepared following incubation of cells under appropriate conditions, or cells were prepared for examination in the hydrated state. Model systems such as synthetic analogues of dense bodies and bacterial polyphosphate granules were prepared by precipitation reactions or by growth of bacteria under appropriate conditions.

2. Electron Microscopic Studies. Electron microprobe techniques or electron energy-loss spectroscopy was done at the BEIB electron microscope facility. Model systems and platelet dense bodies were examined; based on the results obtained, methods were worked out for quantitation of elements of interest.

3. Scanning X-ray Microscopy. Platelets and model systems were examined at the Stanford Synchrotron Radiation Laboratory, utilizing a specially constructed scanning x-ray microscope.

4. Measurement of Mass-Thickness. Platelets, isolated dense bodies, and model systems were studied in a specialized scanning-transmission microscope equipped to record electron scattering profiles. Data was recorded digitally on magnetic tape and transferred to floppy discs. Digital images were displayed and processed on an interactive analysis system.

5. Flash X-ray Microscopy. Platelets and model systems were exposed to nanasecond pulses of long-wavelength x-rays generated by a capacitive-discharge plasma source. Images were recorded on x-ray resist, and after exposure cells were examined by conventional electron microscopy to assess radiation-associated damage.

6. Contact X-ray Microscopy. Photon absorption images were recorded in x-ray resist material, using several-hour exposures to a continuous flux of long-wavelength x-rays. The resist material was developed and studied by conventional electron microscopy.

Major Findings:

1. Electron Microprobe Analysis of Platelets and Model Systems. The core of the human platelet dense body, containing calcium and various phosphate species, does not appear to lose calcium or phosphorous under conditions which cause appreciable loss of serotonin (i.e., biochemical isolation, hypotonic swelling of platelets), and also remains unchanged during incubation of isolated dense bodies at 37°C under conditions which permit the uptake of serotonin.

2. Quantitation of Dense-Body Elemental Composition with Electron Energy-Loss Spectroscopy. Calcium hydroxy-apatite, synthetic calcium phosphates, and synthetic analogues of platelet dense bodies have been used as standards to develop procedures and routines for quantitating calcium, phosphorus, oxygen, and nitrogen by electron energy-loss spectroscopy.

3. Examination of Platelets by Scanning X-ray Microscopy. Human platelets can be imaged utilizing photons in the 300-400 eV range, with a resolution which currently approaches 0.3 μm. In addition, extended atomic fine structure and near-edge fine structure measurements can be obtained for discrete areas in the specimen. Future instrumental improvements should permit a resolution approaching 0.1 μm.

4. Mass-Thickness Measurements of Dense Bodies. Mass-thickness measurements of a population of dense bodies in platelet whole mounts indicates that the within-population variability is sufficiently low to permit detection of a 10% difference in mass thickness with a reasonable sample size. By these criteria, populations of isolated dense bodies have a distribution of mass thickness which is essentially identical to that of dense bodies in intact platelets. Sufficient data has been accumulated to permit estimation of compositional heterogeneity among dense-body populations and evaluation of the relationship between dense body size (membrane area) and mass thickness.

5. Computer Processing of Mass-Thickness Data. Routines have been developed for processing digitally acquired images to derive the mass thickness of selected areas. Work continues on quantifying detection limits and improving programs for processing mass thickness and electron energy-loss data.

6. Flash X-Ray Microscopy. Images of hydrated platelets with limited resolution have been obtained, and work continues on improving the source characteristics to improve resolution and to permit imaging with varying wavelengths (for elemental analysis). Since radiation damage from the intense short burst of photons appears to be related primarily to thermal effects, methods to protect the specimen have been developed.

7. Contact X-Ray Microscopy. The information content of exposed x-ray resists can be greatly increased by two new techniques. First, examination of platelets in the developed resist with the transmission electron microscope shows that dense bodies overlie and appear to be closly associated with a photon-absorbing network extending throughout the platelet cytoplasm. Second, very heavy development of platelet resists shows what appears to be the dense tubular system and that the core material of dense bodies is arranged in a lamellar or filamentous fashion.

Significance to Biomedical Research and the Program of the Institute. This work utilizes a number of ultrastructural approaches to correlate the structure, function, and chemical state of platelet dense bodies. Changes following alterations in constituents have been examined, the relationship between membrane surface area and content has been explored, and possible structural coupling between dense bodies and other cytoplasmic structures has been investigated. Continuation of the work should expand the understanding of factors controlling the storage of amines in dense bodies, and of mechanisms for amine uptake and release.

Publications:

Costa, J.L., Fay, D.D. and McGill, M.: Electron proble microanalysis of calcium and phosphorus in dense bodies isolated from human platelets. Thromb. Res. (in press).

Feder, R., Costa, J.L., Chaudhari, P. and Sayre, D.: Improved detail in biological soft x-ray microscopy: Study of blood platelets. Science. (in press).

Costa, J.L., Smith, M.A., Tanaka, Y. and Cushing, R.J.: Phosphorus and divalent cations in dog, rabbit, and human platelet dense bodies as deduced from electron microprobe studies of air-dried whole mounts. Res. Comm. Chem. Pathol. Pharmacol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00331-03 CN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Use of Nuclear Magnetic Resonance to Study Aminergic Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	R. P. Blumenthal	Chief, LMB	NCI
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	J. A. Ferretti	Physicist, LP	DCRT

COOPERATING UNITS (if any)

LAB/BRANCH
Clinical Pharmacology Branch

SECTION
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INSTITUTE AND LOCATION
NIMH:ADAMHA:Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Nuclear magnetic resonance spectroscopy has been used to study human platelets, chromaffin vesicles, and various phosphate-containing systems which are useful as models for amine storage. In all systems, the motional properties of both amine and nucleotide moieties appear to be related to divalent cation content and to the intra-vesicular volume.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To examine by nuclear magnetic resonance spectroscopy (NMR) the chemical state and motional properties of amine-storing vesicles and of model systems for these structures. The results provide information about the mechanism of amine storage, and the response of the storage system to psychoactive agents.

Studies Implemented: (1) Analysis of the amount and subcellular location of lithium in human platelet (in preparation for NMR of the Li nucleus); (2) evaluation of metabolic pathways in platelets and other systems, and of the relationship of metabolism to amine storage; (3) examination of the motional properties of adenine nucleotides and other types of phosphates in model systems (bacterial granules, liposomes) and chromaffin granules; (4) quantitation of alterations in phosphate motion on the addition of amines or divalent cations.

Methods Employed:

1. General Preparative Procedures.Platelets were isolated from normal volunteers or patients on lithium therapy by differential centrifugation. Bacteria and other prokaryotes were grown by incubation in chemically defined media. Bovine adrenal glands were collected at the slaughterhouse. Chromaffin vesicles were prepared by homogenization and differential centrifugation, and liposomes by sonication of lipids in appropriate media. Cells were incubated according to various protocols and prepared for NMR study by extraction with perchloric acid or by resuspension at a high cell density. Liposomes were concentrated for NMR by column chromatography.

2. Measurement of Lithium in Platelets. Lithium in platelets before and after thrombin treatment was measured by atomic-absorption spectroscopy.

3. NMR Studies. Spectra were obtained of liposomes, chromaffin vesicles, or cells after various prior incubation conditions, and compared with those obtained from cell extracts or with defined preparations containing the molecules of interest.

Major Findings:

1. Localization of Lithium in Human Platelets. Platelets isolated from patients contain appreciable amounts of lithium (about 1% of the total amount of calcium present), which appears to be sequestered predominantly in the amine storage vesicles (dense bodies). Incubation of normal platelets with lithium results in a concentration-dependent uptake of lithium into the dense bodies. The studies indicate that sufficient lithium is present in dense bodies to permit direct NMR examination of the motional properties of the lithium nucleus.

2. Metabolic Pathways in Platelets and Other Systems. When platelets or other cell types are incubated with C^{13}-labelled glucose, some of the labelled material is taken up and converted to glycogen and other sugars. Once metabolism is better understood, its relationship to amine uptake and storage will be examined.

3. Motional Properties of Phosphates in Amine Storing Systems. Although aqueous solutions containing 0.2 M ATP give rise to narrow resonances at characteristic positions, sequestration of the ATP inside liposomes (estimated diameter, 20-25 nm) broadens the observed linewidths and shifts the position of each resonance. Similar changes are seen when chromaffin granules are dehydrated by incubation in hypertonic solutions. Bacterial polyphosphate accumulations give rise to resonances whose linewidths are relatively narrow in the absence of divalent cations, but which broaden considerably following the addition of either calcium or magnesium. When the ratio of magnesium to phosphorus approaches 0.1, the granules assume the motional properties of a solid. Addition of amines sharpens the resonances, as has been observed as well with pig platelet dense bodies. In all three systems, phosphorus linewidths increase with decreasing temperature and thus peak widths as a function of absolute temperature can be used to calculate activation energies for the various types of phosphate aggregates.

Significance to Biomedical Research and the Program of the Institute: Nuclear magnetic resonance has been used to explore the physiochemical properties associated with adenine nucleotides and polyphosphate in model systems and in amine storing vesicles. These studies clarify the relationship between phosphates and amines, and investigate the participation of intra-vesicular complexation in this process. Biomchemical studies suggest that it should be possible to examine as well the effects of metabolism and lithium on amine storage in platelets.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00332-03 CN

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Animal Models for the Study of Drug Effects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert M. Cohen	Staff Physician	CN NIMH
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	Ned H. Kalin	Staff Physician	CN NIMH
	Iain C. Campbell	Visiting Scientist	CN NIMH
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COLLAB:	Leslie F. Major	Chief, Dept. of Psychiatry State Univ. of N.Y., Binghamton, NY	
	Pauline Lerner		

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	0.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations in non-human primates have demonstrated a circadian pattern of change in cerebrospinal fluid prolactin concentrations similar to that previously observed in plasma. Other neuroendocrine substances (e.g., cortisol and β-endorphin) as well as amines and amine metabolites are being studied in relation to neurotransmitter-related drug effects. Measurement of these same substances in cat and rodent cerebrospinal fluid and brain supplement the studies done in monkeys and man. Evidence has been obtained that the neurotransmitter changes induced by the selective monoamine oxidase type A inhibitor clorgyline which has antidepressant properties leads to decreases in the numbers of adrenergic receptors in rat brain. Pargyline, an ineffective antidepressant in our human studies, does not. The temporal pattern of the clorgyline-induced changes in clonidine binding suggests that alterations in presynaptic mechanisms precede those in postsynaptic sites.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Rhesus monkeys, cats and rodents are utilized to study the effects of various drugs on cerebrospinal fluid and brain concentrations of neurotransmitters, their metabolites and their receptors and of neuroendocrine substances to better understand the results obtained from cerebrospinal fluid studies in humans.

Methods Employed:

1. Biological assessment: Cerebrospinal fluid, plasma and platelets are collected for the measurement of hormones, amines and their metabolites, enzyme activities, other chemical variables, and drug levels. Cerebrospinal fluid (CSF) is collected by means of cisternal taps and in some cases by means of indwelling ventricular or lumbar cannulas for continuous flow into a -70°C fraction collector over a 24-hour period.

Receptors from crude brain homogenates are measured by standard radioactive ligand assays. [^{3}H]-dihydroalprenololol, [^{3}H]-WB4101 and [^{3}H]-clonidine are the specific ligands used for the measurement of β-, α_1-, and α_2-receptors respectively.

2. Behavioral assessment: Detailed rating scales of activity, arousal, posturing, appetite, grooming, and aggressiveness were employed both prior to and during treatment with various neurotransmitter-related drugs.

Major Findings:

Studies of Hormonal Diurnal Variation: In studies coordinated by Dr. Kalin, the 24-hour CSF immunoreactive prolactin rhythm for four rhesus monkeys was established. The rhythm is characterized by episodic changes which begin to increase 1-2 hours after darkness and has two peaks, one at 0200 (+33% from mean) and a second peak at 0500 (+29.6%). Shortly after lights come on, values fall below baseline. All daytime values are less than the 24-hour mean. A repeated measures analysis of variance demonstrated a statistically significant difference over time with a peak of 6.3 $\pm$ 2.5 ng/ml (0200) and an approximate 2-fold peak-to-trough difference with a mean of 3.2 $\pm$ 0.7 ng/ml of prolactin immunoreactivity.

This appears to be the first report of a significant diurnal variation in the CSF of an anterior pituitary peptide hormone, and appears similar to that previously reported for human plasma.

Receptor Studies: The potential importance of neurotransmitter level changes to the overall functioning of the CNS catecholaminergic system were assessed by receptor studies in rat brain. 1 mg/kg clorgyline and 1 mg/kg pargyline were administered daily over a 3-week period. Only clorgyline produced decreases in β-receptor number. As clorgyline, but not pargyline at these doses produced increases in norepinephrine levels, it appeared that these changes were secondary to increases in synaptic norepinephrine. In

following the time course for the β-receptor changes which became significant at 10 days, these were observed to be preceded by earlier (3 days) decreases in the α_2-receptor. The change in α_2-receptor binding may represent changes in the "autoreceptor" partially mediating subsequent increases in norepinephrine in the synapse. The physiological correlates of these receptor changes are under current investigation.

Neurotransmitter CSF Studies: In an attempt to bridge the gap between human studies where only bodily fluids (CSF and plasma) are studied, and rodent studies in which only plasma and brain tissue are studied, cat plasma and CSF were examined both acutely and chronically and brain tissue analyzed at the termination of a drug trial with clorgyline and pargyline. Aminergic changes in CSF over the course of the 3-week study were followed and then compared to those found in the brain upon conclusion of the study. CSF DBH activity, which is known from our studies in patients to be reduced after four weeks' treatment with MAO-inhibiting antidepressants, was found to not change significantly until 18-21 days of treatment with the MAO inhibitors, approximately the time at which clinical response to these drugs is usually seen. These findings suggest that the clinical response to MAOI's is related not to simple inhibition of amine degradation in the aminergic systems, but to later, adaptational alterations, such as receptor changes. In addition, consistently higher ratios of norepinephrine to DBH activity in CSF were found in the cat, as in the human. This change, which most likely reflects a reduction in central noradrenergic firing rates, is most likely reflective of a change in presynaptic receptor sensitivity.

Publications:

Lerner, P., Major, L.F., Dendel, P.S., Campbell, I.C., and Murphy, D.L.: Central and peripheral dopamine beta hydroxylase: Responses to long term treatment with monoamine oxidase inhibitors. Neuropharmacology 19:877-882, 1980.

Lerner, P., Dendel, P.S., and Major, L.F.: Dopamine-β-hydroxylase in cerebrospinal fluid and plasma: Effects of α-adrenergic agents. Brain Res. 189:183-191, 1980.

Kalin, N.H., Insel, T.R., Cohen, R.M., Risch, S.C., and Murphy, D.L.: Diurnal variation in CSF prolactin concentration of the rhesus monkey. J. Clin. Endocrin. Metab. 52:857-858, 1981.

Cohen, R.M., Campbell, I.C., Dauphin, M., Tallman, J.F., and Murphy, D.L.: Changes in alpha and beta-receptor densities in rat brain as a result of treatment with monoamine oxidase inhibition (MAOI) antidepressants. Neuropharmacology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00333-02 CN

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Neuropharmacology and Psychobiology of Alcoholism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Elliot Shullman	Neurologist, Washington VA Hospital	
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COOPERATING UNITS (if any)

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Psychiatry Service, NNMC

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project has been discontinued.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00334-03 CN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of the pressor, metabolic and behavioral responses to intravenous tyramine.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	D. C. Jimerson	Staff Psychiatrist	LCS NIMH
COLLAB:	C. R. Lake	Staff Psychiatrist	BP USUHS

COOPERATING UNITS (if any)

LAB/BRANCH
Clinical Neuropharmacology Branch

SECTION
NIMH:ADAMHA:IR:DCBR

INSTITUTE AND LOCATION
NIMH:ADAMHA:Bethesda, Md. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An increase in sensitivity to the pressor effects of tyramine, an indirectly acting sympathomimetic agent, is a well known side effect of MAO inhibitor treatment. Using an intravenous tyramine steady state infusion technique we have studied the effects of the selective inhibition of the A and B forms of MAO on tyramine's pressor effects in patients treated with clorgyline, pargyline and deprenyl. After four weeks of treatment, clorgyline produced a significantly greater increase in tyramine sensitivity in comparison to a medication-free baseline (29-fold) than did pargyline (12-fold) or deprenyl (1.7-fold). This data suggests that the tyramine sensitivity which develops during MAO inhibitor administration is principally due to a reduction in MAO type A activity. A high correlation observed between changes in tyramine sensitivity and reduced plasma 3-methoxy,4-hydroxy phenylglycol concentrations supports this interpretation. Clorgyline administration was also found to be associated with reductions in tyramine-stimulated plasma norepinephrine release.

Project Description:

Objectives: The purpose of this project is to evaluate the comparative effects of selective MAO inhibitors on the pressor response to tyramine and to evaluate further the biochemical and physiological effects of tyramine infusions. Since selective MAO inhibitors have proven useful in treating depression and hypertension, as well as adjuncts in the treatment of Parkinson's disease, an understanding of their effects on tyramine sensitivity is of great importance. As an indirectly acting sympathomimetic, tyramine is useful for the clinical evaluation of peripheral noradrenergic function.

Methods Employed:

An intravenous steady state tyramine pressor response test is administered to drug free patients and again during the course of treatment with one of the following selective MAO inhibitors: clorgyline (a selective MAO-A inhibitor), pargyline (a partially selective MAO-B inhibitor) and deprenyl (a more selective MAO-B inhibitor). The purpose is to achieve a 30 mm Hg rise in systolic blood pressure and to maintain this elevation for 5-8 minutes. Careful calculation of dosage and rates of infusion permit safe infusions both on and off the MAO inhibitors. The amount of tyramine per minute which maintains the pressor endpoint is the dose endpoint, and provides an index of tyramine pressor sensitivities for comparisons between subjects and treatment periods. Blood sampling is accomplished prior to and during the infusion. Samples are analyzed by enzyme isotope assay for catecholamines and by gas chromatography--mass spectroscopy techniques for MHPG.

Major Findings:

The enhanced sensitivity to the pressor effects of tyramine, an indirect-acting sympathomimetic found abundantly in the diet, is a well known potentially dangerous side effect occurring during treatment with commonly used non-selective MAO inhibitors. The effects of treatment with the selective MAO-A inhibitor, clorgyline, and the partially selective MAO-B inhibitors, pargyline and deprenyl, on tyramine's pressor effects were studied in depressed patients using an intravenous steady state tyramine infusion technique. After four weeks of treatment, clorgyline produced a significantly greater increase in tyramine sensitivity in comparison to a medication-free baseline (29-fold) than did pargyline (12-fold) or deprenyl (1.7-fold). The pressor effects of tyramine were significantly prolonged after cessation of the infusion during both clorgyline and pargyline but not deprenyl treatment. These data from intravenous tyramine administrations suggest that intestinal MAO inhibition is not the major determinant of the enhanced tyramine pressor sensitivity produced by clorgyline and pargyline.

The relationship between changes in intravenous tyramine pressor sensitivity accompanying selective MAO inhibitor treatment and estimates of MAO-A and MAO-B inhibition *in vivo* were also studied. Reductions in

platelet MAO activity provided an index of MAO-B inhibition, while changes in plasma MHPG were used as an hypothesized reflection of MAO-A inhibition. Chronic treatment with the MAO-A inhibitor, clorgyline, and the MAO-B inhibitor, pargyline, showed significant inhibition of the alternate MAO enzyme as well, although this crossover effect was greater for pargyline than for clorgyline. The MAO-B inhibitor, deprenyl, appeared to maintain the greatest degree of MAO inhibition selectivity in vivo. Tyramine pressor sensitivity changes accompanying the administration of the MAO inhibitors were highly correlated with decreases in plasma MHPG ($r = .92$), supporting the data indicating the rank order of clorgyline>pargyline> deprenyl for enhancement of tyramine pressor sensitivity. We have thus concluded that tyramine potentiation is primarily a function of MAO-A rather than MAO-B inhibition in man.

The 30 mmHG rise in systolic blood pressure produced by the tyramine infusion was associated with a significant increase in plasma NE at the pressor endpoint. Individual subject changes in plasma NE from resting to pressor endpoint were highly correlated with resting plasma NE levels. Resting plasma NE levels were also significantly correlated with resting levels of plasma MHPG and with tyramine dose endpoints. One interpretation of these changes with chronic MAO-inhibitor treatment is that clorgyline induces a down-regulation of presynaptic adrenergic activity.

Publications:

Pickar, D., Cohen, R.M., Jimerson, D.C., and Murphy, D.L.: Tyramine infusions and selective MAO inhibitor treatment. I. Changes in pressor sensitivity. Psychopharmacology (in press).

Pickar, D., Cohen, R.M., Jimerson, D.C., Lake, R.L., and Murphy, D.L.: Tyramine infusions and selective MAO inhibitor treatment. II. Interrelationships among pressor sensitivity changes, platelet MAO inhibition and plasma MHPG reduction. Psychopharmacology (in press).

Pickar, D., Lake, C.R., Cohen, R.M., Jimerson, D.C., and Murphy, D.L.: Alterations in noradrenergic function during clorgyline treatment. Commun. Psychopharmacol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00335-03 CN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Smooth Pursuit Eye Tracking Impairment and Its Relation to Psychopathology and CNS Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Larry J. Siever	Staff Fellow	CN NIMH
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	Monte S. Buchsbaum	Section Chief	BP NIMH
	Theodore P. Zahn	Research Psychologist	LPP NIMH
	Douglas Reingold	Biologist	CB NEI
	Peter Bernad	Visiting Scientist	CN NINCDS
	Susomo Sato	Section Chief	CN NINCDS

COOPERATING UNITS (if any)

Department of Psychology,
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LAB/BRANCH
Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.4	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project aims to characterize psychological and psychobiological attributes that are associated with alterations in smooth pursuit eye movements. The smooth pursuit system regulates the eyes' smoothly following a moving target and can be measured by an electro-oculograph recording of an individual watching a moving pendulum. By using the high-risk strategy of screening a large group of college student normal volunteers, individuals with various alterations in smooth pursuit patterns have been identified. Individuals with two common types of alterations have been compared with those with more accurate tracking using a variety of psychological and biological tests to explore the relationship of these patterns to personality, psychophysiological and biochemical variables.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project is designed to study individual variations in smooth pursuit eye movements and their relationship to cognitive controls and personality tendencies. In order to understand such relationships, we are employing the "biological high risk strategy," i.e., identifying individuals from a population of normal volunteers with extreme values of a biologic variable implicated in the etiology of psychiatric disorders and comparing them psychologically with volunteers with normal values for that variable. This strategy has been employed successfully in studies of monoamine oxidase levels in man. College student normal volunteers have been screened to determine the quality of their smooth pursuit tracking. Subgroups of these students showing definite alterations in their smooth pursuit patterns have been compared to those with extremely accurate tracking on a variety of psychological, biochemical, and psychophysiological tests.

Methods Employed:

Volunteers from a local college, after giving informed consent, participated in a screening session testing their smooth pursuit eye movements. They were instructed to follow a moving pendulum, which made a target excursion of 20 degrees of visual angle and oscillated at 0.4 Hz. Their eye movements were measured via surface electrodes by an electro-oculograph and both eye position and velocity were recorded on paper.

These records were scored for velocity arrests, i.e., points at which the eye stops moving while the target continues, and the pattern of deviation of the eye movements. For example, there are individuals with (1) a high velocity arrest score and "spikey" pattern of eye movements, as well as (2) individuals with difficulty initiating eye tracking, so that saccades replace the smooth pursuit movements, i.e., "saccadic substitution." A group of individuals from each of these two groups was compared with a matched control group exhibiting exceptionally good tracking patterns using a variety of psychological and biological tests as well as a clinical interview and family history.

Major Findings:

Two hundred eighty volunteers have been screened and the accuracy of their smooth-pursuit eye movements assessed. Twenty individuals with low accuracy tracking, twenty individuals with high accuracy tracking, and ten with "saccadic substitution," have been identified and have undergone extensive psychologic testing including Wexler Adult Intelligence Scale, Rorschach, Minnesota Multiphasic Inventory, and a variety of cognitive and perceptual tests. Most of this group of 50 have come to the NIH Clinical Center for psychophysiologic studies with Dr. Buchsbaum (Continuous Performance Task), Dr. Zahn (reaction time), neurological exam and EEG with Dr. Brody or Dr. Bernad, and extensive psychiatric interviews including the Schedule for Affective Disorder and Schizophrenia (SADS) and family history with Dr. Siever.

Low accuracy trackers showed evidence of more psychosis-related traits than high accuracy trackers in the psychiatric interview conducted by staff blind to their eye-tracking status. Nine of 11 individuals satisfying DSM-III criteria for Schizotypal Personality Disorder were in the low accuracy tracking group, as were all eight individuals with a history of hypomania by Research Diagnostic Criteria (RDC). Schizophrenia-related characteristics were found in 21/31 of the low accuracy trackers. Further correlations with clinical ratings indicated significant associations between degree of accuracy of tracking and characteristics of flattened affect, decreased rapport, referential ideas, suspiciousness, brief delusions, social isolation, dependency, and preference for being alone. Low accuracy tracking also correlated with the number of neurological soft signs, abnormalities in EEG, delayed visual evoked potentials, and left-handedness, although most of these results were within clinically normal limits. Correlations also emerged between tracking inaccuracy and the Chapman anhedonia scale, embedded figures test, and other psychologic test data. Results from this study are still being analyzed, but suggest that low accuracy tracking is associated with schizophrenia-related and mania-related traits. In these subjects with schizophrenia-related traits, the results reflected the profile of an individual with subtle neurologic dysfunction, poor perceptual boundary differentiation, decreased affect, and social isolation with avoidance of interpersonal intimacy. We are also studying the correlation between baseline inaccuracy of tracking and behavioral response to amphetamine in psychiatric patients, to test the hypothesis that arousal may enhance psychosis-related behaviors in these vulnerable individuals. Preliminary evidence from a study with Dr. John Nurnberger (BPB) suggests that bipolar low accuracy trackers show a greater elation response to amphetamine. We also testing schizophrenia and obsessive-compulsives in this paradigm.

Finally, we are evaluating correlations between inaccuracy of tracking, psychosis-related characteristics and other biologic variables in patient groups including obsessive-compulsives, affectively disordered patients, and schizophrenics in collaboration with Dr. Nurnberger, Dr. Thomas Insel (CNB), Dr. Daniel van Kammen (BPB), and others.

Significance to Biomedical Research and the Program of the Institute: The smooth pursuit system has been a focus of interest in psychiatry since it has been demonstrated that it is significantly impaired in psychotic individuals, particularly schizophrenics, as well as some patients with severe affective disorders. This is hypothesized to represent a deficit in involuntary attention or cognitive centering. "Good" and "poor" tracking patterns have been shown to be under genetic control and thus to represent one of the few genetically determined biologic variables consistently associated with schizophrenic and other psychoses. By using the high-risk strategy of screening a college population for alterations in this system, the relationship of this variable to qualities of attention and personality tendencies in a non-psychiatrically defined population might be clarified without the

confounding variables of the length of illness, medication or hospitalization. This may facilitate delineating a dimension of psychological function closely tied to involuntary attention as reflected in eye-tracking performance.

Proposed Course: We will continue to study the 50 selected volunteers until as many as possible have undergone psychophysiological and neurologic testing, a psychiatric interview, and repeat eye-tracking evaluation. In addition, blood is being drawn for monoamine oxidase and dopamine-β-hydroxylase activity measurements. We are also continuing to study schizophrenic, obsessive-compulsive and affective ill patients.

Publications:

Siever, L.J., Coursey, R.D., Alterman, I.S., Buchsbaum, M.S., and Murphy, D.L.: Psychological and physiological correlates of variations in smooth pursuit eye movements. In Usdin, R. and Hanin, I. (Eds.): Biological Markers in Psychiatry and Neurology. New York, Pergamon Press (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00336-02 CN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Phenomenology and Treatment of Obsessive-Compulsive Disorder in Adults

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas R. Insel	Staff Physician	CN	NIMH
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	Dennis L. Murphy	Chief	CN	NIMH
COLLAB:	Larry J. Siever	Staff Physician	CN	NIMH
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Clinical Neuropharmacology Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.4	0.9

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Obsessive-compulsive disorder is an uncommon syndrome about which little is known. The purpose of this study is to collect basic data on the phenomenology, and treatment of this disorder. Such biologic measures as sleep, monoamine metabolites, averaged evoked potentials, and galvanic skin response are being studied in relation to symptoms and behavior in these patients. Treatment with clorgyline, a selective MAO inhibitor, chlorimipramine, a tricyclic antidepressant and placebo are being compared.

PHS-6040
(Rev. 10-76)

Project Description: ZO1 MH 00336-02 CN

Objectives: The current study addressed four different sorts of questions. The first focuses on the phenomenological question: Is this a heterogenous syndrome and, if so, how can we identify different subtypes? Related issues are as follows: What is the relationship of objectively measured anxiety to symptoms e.g., do patients with rituals have more or less anxiety after performing their compulsive behavior? Is there a cyclic rhythm to the symptoms as has been described in the stereotypes of schizophrenics? A second set of questions relates to the neurophysychology of this disorder: Do some of these patients show abnormal findings on neurological exam, cognitive testing, laterality tasks, or CAT scans? How might such patients differ symptomatically from those without neuropathological findings? A third question deals with etiology, namely, are there genetic markers that correlate with the phenotypic expression of the disorder? This is particularly of interest in families where multiple individuals are affected. Finally, there is the neuropharmacologic question: Is clorgyline as effective as chlorimipramine in treating obsessive-compulsive disorder, and if so, is its effectiveness correlated with the biological measures obtained?

The purpose of the present investigation, then, is to develop a more comprehensive picture of the phenomenology, the neuropsychology, the genetics, and the neuropharmacology of this syndrome.

Methods Employed:

Obsessive-compulsive subjects are studied in both inpatient and outpatient settings. Patients who apply for the protocol are carefully screened with an extensive psychological and medical battery. Those accepted must have had obsessive or compulsive symptoms for at least one year independent of another psychiatric diagnosis. An initial evaluation includes cognitive, projective, psycholinguistic and psychophysiologic (EEG, ESR) testing. Biologic measures which have proved useful in studies of affective or schizophrenic disorder are focused on here; sleep physiology, average evoked response, urinary and cerebrospinal fluid monoamine metabolites, a variety of platelet and RBC enzymes, and the dexamethasone suppression test are all studied. Cerebral CT scans are done to investigate the possibility of gross neuropathology in this disorder. A major aspect of the study is the comparative double blind drug trials. These trials follow a randomized cross-over design with assessment by both self and observer ratings on a weekly basis. Specific biological and psychological measures are repeated during the various stages of the drug trials for correlation with clinical state.

An additional study begun within the last year is examining amphetamine behavioral and neuroendocrine responses as predictors of clinical response to chlorimipramine or clorgyline. The theory, borrowed from analogous studies in depressed patients, has been that those patients with activation or euphoria responses to a single dose of d-amphetamine would be more likely to respond thera- peutically to one of the drugs in the double blind crossover study.

Major Findings and Proposed Course:

Five patients have completed the double blind crossover trial. Five more are currently involved in the study. An additional five have been involved in serious parts of the assessment battery but have not participated in the lengthy crossover medication trial. The data from this pilot group are currently being analyzed.

A preliminary view of the data suggests findings in a number of areas of study. Specifically, the psycholinguistic, neuropsychologic, and psychophysiologic testing has shown (a) marked variability within the group, and (b) surprising deficits in a subset of one cohort. Similarly, sleep EEG and dexamethasone suppression findings suggest that some primary obsessional patients appear indistinguishable from patients with primary affective disorder on these biological parameters. We are currently reviewing the spectrum of tests to describe a taxonomy of the obsessional syndrome. Our current view is that the symptoms represent a final common pathway leading from various neurological and psychological sources.

The preliminary findings from the treatment part of the study are quite interesting. Medication does seem to be of substantially greater benefit than placebo for most of the 10 patients in the crossover study. However, there is no clear evidence at this point that clomipramine is superior to clorgyline. Side effects of clorgyline, particularly hypotensive effects, have been much less than with our depressed patients. Age may explain the difference. The only major adverse effects have been with two patients who developed myoclonus after a first does of clomipramine. Both of these patients had received clorgyline four weeks earlier, suggesting that this rare side-effect may have resulted from an interaction with the MAO inhibitor.

In the coming year we will be adding more subjects to both the medication trial and the assessment study. We will also continue the data analysis of the current cohort with the following questions in mind. Are the medication effects mainly related to changes in obsessions, mood, or anxiety level What predicts medication response Does clinical response to chlorimipramine correlate with plasma levels of drug or drug metabolites Are patients with neuroendocrine and sleep changes suggestive of depression more likely to respond to medication As Obsessive Compulsive Disorder is an uncommon syndrome, we feel that even with a cohort of less than 30 patients we can make substantial contributions to both the theoretical understanding of the symptoms and the strategy for treatment.

Publication:

Insel, T., and Murphy, D.L.: The psychopharmacologic treatment of obsessive-compulsive disorder: A review. J. Clin. Psychopharm. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00337-02 CN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Neuropharmacology of Neuroendocrine Regulatory Mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Ned H. Kalin	Staff Physician	CN NIMH
	Samuel C. Risch	Staff Physician	CN NIMH
	Thomas R. Insel	Staff Physician	CN NIMH
	David Pickar	Chief, Unit on Drug Abuse	BP NIMH
COLLAB:	Gary W. Kraemer	Investigator, Wisconsin Primate Lab., Univ. of Wisconsin	
	William T. McKinney	Professor, Dept. of Psychiatry, Univ. of Wisconsin	

COOPERATING UNITS (if any)

Wisconsin Primate Laboratory, University of Wisconsin

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Clinical Neuropharmacology Branch

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	1.5	1.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have continued to characterize the regulatory mechanisms related to hypothalamic-pituitary activity in rhesus monkeys and man. (1) The extent of cortisol suppressibility in an individual monkey in response to a low dose of dexamethasone has been demonstrated to be reproducible over time. (2) A positive relationship has been established between the extent of dexamethasone suppressibility and behavioral arousal. (3) The physostigmine behavioral syndrome is accompanied by increases in plasma ACTH which positively correlate with previously observed plasma beta-endorphin increases but not with cortisol elevations. (4) The current knowledge of the neuropharmacology of neuroendocrine regulatory mechanisms in affective disturbances and their assessment by pharmacological challenge strategies was formally reviewed.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The limbic-hypothalamic-pituitary axis plays an important role in the response to stress in both animals and man. In addition, dysregulation of this axis has been demonstrated in some depressed patients. This project is investigating the underlying neurotransmitter-related mechanisms of regulation in this hormonal axis. The study of these mechanisms has a potentially explanatory role in the understanding of animal models of stress vulnerability, including those related to the production of depression in monkeys. These models, some of which are based on neuropharmacologic challenges which can also be carried out in man, may also provide direct insights into human affective disorders. Rhesus monkeys can be usefully employed to evaluate hormone responses in the CSF, which is helpful in the evaluation of the significance of plasma hormone responses for central nervous system function.

Methods Employed:

Neuroendocrine Measurements: Monkey cerebrospinal fluid is collected by means of indwelling lumbar cannulae for continuous flow into a refrigerated fraction collector. Monkey plasma is obtained either by use of indwelling venous catheters or by femoral venipuncture following ketamine-induced anesthesia. Human samples are obtained by maintenance of a heparin lock in the subject's forearm, allowing frequent sampling with minimal stress. Cortisol, growth hormone, prolactin, ACTH, and β-endorphin are assayed by competitive binding utilizing measured displacement of labelled competitive binding tracers from their specific antibodies. Biologically active opioid peptides are measured by competition with (H^3)-$(D\text{-}Ala)^2$ enkephalin-L-(leu-amide) for binding sites on crude rat brain membranes.

Psychological and Behavioral Assessment: Pretreatment evaluation of patients and volunteers is accomplished through interviews of the individual and family members as well as assessment of past medical records. When appropriate, structured Affective Disorders Interviews (SADS) are used and Research Diagnostic Criteria (RDS) diagnoses made. Direct behavioral observations are performed by both nursing staff and physicians by the use of quantitative rating scales. Subjects themselves fill out appropriate ratings. These approaches enable us to attempt to correlate specific neuroendocrine findings with diagnostic categories and/or behavioral changes observed spontaneously or in response to pharmacologic challenges or antidepressant treatments.

Major Findings:

Dexamethasone Suppression Studies: Dr. Kalin, in collaboration with Drs. Kraemer and McKinney, has continued to explore the dexamethasone suppression test as a tool for the investigation of nonhuman primate models of depression and stress. As previously reported, rhesus monkeys were suppressed in a dose-dependent fashion at doses between 0.75 and 33 μg/kg. A replicative study performed five months later at a single dose of dexamethasone (17 μg/kg, a dose normally producing maximal cortisol suppression) resulted in a highly correlated percent cortisol suppression for individual

animals ($r = 0.91$, $p < 0.005$). This suggests that dexamethasone suppressibility may be a stable characteristic of individual animals.

In a separate group of animals, dexamethasone suppressibility correlated significantly ($r = 0.75$, $p < 0.025$) with individual mean arousal ratings in response to the stressful procedure of nasogastric tube insertion conducted daily over three months. Baseline cortisol levels in these same animals did not correlate with the same arousal ratings. Taken together, these results suggest that an individual's hypothalamic-pituitary glucocorticoid feedback system may be closely linked with behavioral arousal. Thus, further assessment of this system may lead to important information concerning immediate and adaptive responses to stress.

The Physostigmine Syndrome: Coordinated by Dr. Risch, we have continued to analyze the neuroendocrine correlates of cholinergically initiated depressive affect through the use of intravenous physostigmine (0.02 µg/kg) infusions. As previously reported, this mixed nicotinic-muscarinic agent causes increases in plasma cortisol and beta-endorphin immunoreactivity levels; however, only the latter correlated with individual behavioral changes. Recently, we have observed a significant correlation between the rise in beta-endorphin/beta-lipotropin immunoreactivity and plasma ACTH ($r = 0.85$, $p < 0.01$). This result supports the co-release of β-endorphin and ACTH as has been reported by others under varying circumstances. A lack of proportionability in ACTH release and cortisol secretion ($r = 0.39$) in man was also observed. These findings provide another link between the "physostigmine syndrome" and "depression," as hypersecretion of cortisol, presumably secondary to increased pituitary ACTH release, is a common abnormality in endogenous depression in man.

Review of Affective Challenge Strategies: The current knowledge of neuroendocrine functioning in affective disturbances as assessed by pharmacologic challenge strategies was formally reviewed. The purpose of this review was to arrive at some conclusions as to the current context of challenge studies in the diagnosis of and in the understanding of the etiology of mood disturbances. The data, based on peptide challenges (e.g., TRH) and catecholamine challenges (e.g., clonidine) in addition to those of dexamethasone challenges, strongly suggest rather profound alterations in the hypothalamic-pituitary axis in affective illness. The challenge strategy has become an important tool in the diagnosis and typing of mood disordered patients of all primary diagnoses and is currently part of our own assessment battery. We continue to pursue studies both in depression and obsessive-compulsive illness of clonidine and amphetamine responses as well as dexamethasone responsiveness.

Publications:

Kalin, N.H., Risch, S.C., Cohen, R.M., Insel, T., and Murphy, D.L.: Dexamethasone fails to suppress β-endorphin plasma levels in rhesus monkeys and man. Science 15:827-828, 1980.

Risch, S.C., Cohen, R.M., Janowsky, D.S., Kalin, N., and Murphy, D.L.: Plasma β-endorphin and cortisol elevations accompany the mood and behavioral effects of physostigmine in man. Science 16:29-31, 1980.

Kalin, N.H., Cohen, R.M., Kraemer, G.W., Risch, S.C., Shelton, S., McKinney, W.T., Cohen, M., and Murphy, D.L.: The dexamethasone suppression test as a measure of hypothalamic-pituitary feedback sensitivity and its relationship to behavioral arousal. Neuroendocrinology 32:92-95, 1981.

Naber, D., Cohen, R.M., Pickar, D., Kalin, N.H., Davis, G., Pert, C.B., and Bunney, W.E., Jr.: Episodic secretion of opioid activity in human plasma and monkey CSF: Evidence for a diurnal rhythm. Life Sci. 28:931-935, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00338-01 CN

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Families of Origin in Obsessive-Compulsive Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carol F. Hoover	Social Worker	CNB NIMH
PI:	Thomas Insel	Staff Physician	CNB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Families of severely obsessive-compulsive patients showed characteristic similarities in relationship and interaction, suggesting that the family milieu may be crucial in the development of obsessional symptoms. These symptoms were used by the patient to enslave the family and to erect a powerful barrier against parental expectations of symbiosis.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To evaluate the family context of obsessive-compulsive illness.

Methods Employed:

Individual and conjoint interviews of 10 severely obsessive-compulsive patients and members of their families.

Major Findings:

The families of these young obsessive-compulsive adults were characterized by:

(a) Disappointing marriages and sexual conflicts of parents.
(b) Projection of unfulfilled marital closeness needs upon the child.
(c) Preponderance of symbiotic needs emanating from one parent, often the father, yielding an "unbalanced triangle" in relationship.
(d) Erection of symptomatic barriers to those needs by the child.
(e) Compulsive traits in parents which prevented early recognition of child's abnormal course.
(f) Family subjection to patient tyranny based on parental closeness needs.
(g) Overwhelming parental guilt and acknowledgement of inability to say "no" to patient's demands, no matter how bizarre.
(h) Mutual entrapment in a family isolated from community life, with inordinate ties to the grandparent generation.

The patient feared to relinquish his tyranny over the parents, but longed for escape from the power of his symptoms. Parents found it hard to let go of symbiotic expectations, while longing for release from their enslavement.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00446-12 CP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Inpatient Clinical studies of Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Frederick Goodwin	Chief	CP/NIMH
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	Yolande Davenport	Chief, Unit on Family Studies	CP/NIMH
	Philip Gold	Chief, Unit on Neuroendocrinology	CP/NIMH
	William Potter	Asst. to the Branch Chief	CP/NIMH
	Rex Cowdry	Chief, Outpatient Unit	CP/NIMH
	Steven Paul	Chief, Unit on Preclinical Pharmacology	CP/NIMH
	Alfred Lewy	Guest Worker	CB/NEI
	Markku Linnoila	Guest Worker	CP/NIMH
	Norman Rosenthal	Clinical Associate	CP/NIMH
	Richard Ross	PRAT Fellow	CP/NIMH
	Leslie Becker	Clinical Associate	CP/NIMH
	Richard Hauger	PRAT Fellow	CP/NIMH

COOPERATING UNITS (if any)
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LAB/BRANCH
Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.3	2.5	1.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this integrated group of research studies is a more comprehensive understanding of the pathophysiology of unipolar and bipolar affective disorders, particularly with regard to the complex interrelationships among clinical phenomenology, diagnosis, biological variables, and pharmacologic response. The studies are both cross-sectional and longitudinal; drug trials are double-blind. Integration of the inpatient and outpatient units has advanced, focusing on a brief pre-treatment inpatient evaluation of patients with a variety of endogenous and non-endogenous depressive syndromes, followed by outpatient treatment. Meanwhile, longitudinal inpatient studies of endogenous depression, mania, and rapid-cycling bipolar illness continue. Major projects include the study of circadian and seasonal rhythms, neuroendocrine substances and monoamine neurotransmitters and their metabolites, both during untreated affective episodes and following specific treatment. The primary treatment modalities include 1) environmental manipulations, such as sleep deprivation or sleep shifts, 2) tricyclic antidepressants showing specificity for given neurotransmitter systems, and 3) electroconvulsive therapy (ECT).

Project Description:

This description attempts to provide a comprehensive view of the inpatient clinical studies of manic-depressive illness. In this group of studies the overall objective is a more comprehensive understanding of the pathophysiology of unipolar and bipolar affective disorders, particularly in relation to specificity of diagnosis, existence of clinically or biologically identifiable subgroups, the nature of predisposing factors, and the interrelationship between pharmacological and psychodynamic factors in clinical improvement.

Traditionally, the focus of our specialized resources has been the longitudinal study of episodes of affective illness, before, during and often following a variety of therapeutic interventions. Initially, studies focused on classical, discrete episodes of mania or endogenous depression; more recently, our inpatient resources have been devoted increasingly to individuals with rapid-cycling biopolar illness. During the past year we continued to expand the "brief inpatient evaluation" program for depression, in which outpatients are admitted to the unit for one week of pre-treatment studies and are then discharged to be treated as outpatients (see Project Z01 MH 018523-03 CP). This approach enables us to evaluate a much broader spectrum of depressive disorders, using the methodologies previously applied to the endogenous depressions, without seriously compromising our ongoing longitudinal studies of entire illness episodes. Both the total number of patients studied and number of individual projects has increased. The resulting impact on the ward milieu has been well managed.

Central to an effective program of inpatient studies are regular and reliable procedures for gathering data. A variety of variables are monitored continually during a patient's hospital stay. Trained nursing staff members complete twice-daily global ratings, assessing depression, mania, psychosis, anger, and anxiety. Twice a week a modified form of the Brief Psychiatric Rating Scale (BPRS) is scored for each patient. Patients complete self-rating forms twice a day. Specialized scales, such as the Hamilton Depression scale, the Beck Depression Inventory, or the Symptom Check List (SCL-90), are used for particular studies. Information regarding families is obtained through ongoing contact between the social workers and the patient's relatives.

Behavioral data are continually collected and quantified. Sleep is recorded at half-hour intervals throughout the night. Motor activity is recorded continously using solid-state wrist activity monitors (See Project Z01 MH 00450-07 CP), allowing the application of sophisticated mathematical techniques such as spectral analysis to define hitherto unrecognizable patterns in the data. Finally, samples of plasma and urine are collected at regular intervals and stored for subsequent analysis, providing a "bank" of biochemical specimens from various mood states and treatment.

In addition, specific cross-sectional studies are scheduled to provide information about the patient's pre-treatment biochemical state and the biochemical changes associated with changes in mood and treatment modality.

Among these cross-sectional studies are: baseline neuroendocrine values, circadian neuroendocrine patterns, and responses to neuroendocrine challenges (dopamine, saline, and TRH infusions) (See Project Z01 MH 00452-06 CP); circadian patterns of activity and temperature (See Project Z01 MH 00450-07 CP); sleep recordings; and cerebrospinal fluid and urinary monoamines and monoamine metabolites (See Project Z01 MH 00447-12 CP).

Since descriptive and diagnostic dimensions are crucial to the meaningful interpretation of psychobiologic data, work on the standardization of these dimensions has continued. Use of the Research Diagnostic Criteria (RDC) (described in detail in the July 1976 - September 1977 report on Project Z01 MH 00446-08 CP) is now routine. Discharge review conferences are used to establish both a final RDC diagnosis and assess the extent to which each patient has "typical endogenous" depressive episodes. In order to facilitate this task a subjective "typicality" scale is asessed and a record of specific endogenous features is made.

After the evaluation phase of the hospitalization, a variety of investigational interventions are employed. Environmental manipulations such as sleep shifts and exposure to bright light, have played an increasingly prominent role in the overall research program.

A variety of pharmacologic studies continue on the inpatient unit. All drug trials are conducted under double-blind conditions with alternating active and placebo periods in a non-random design. The use of placebo substitution following active drug increases one's level of confidence in the efficacy of a compound in an individual patient. This strategy is important in the evaluation of drug effects because of the frequency of spontaneous remissions and exacerbations in manic-depressive illness.

An extension of previous studies of the behavioral and biochemical effects of electroconvulsive therapy (ECT) is under way, with increased emphasis on neuroendocrine and circadian parameters.

Results of these interventions are described in greater detail in the individual project reports.

Finally, "normal volunteers" live on the inpatient unit for varying periods of time to participate in specific investigational studies. During the past year, they have been involved in studies of the effects of light and of sleep shifts on neuroendocrine functioning and various circadian rhythms (See Project Z01 MH 00450-07 CP) and in studies of the biochemical, physiological, and behavioral effects of desipramine and of DDAVP (a vasopressin analog).

Publications:

Zis, A.P., Grof, P., Webster, M. and Goodwin, F.K.: Prediction of relapse in recurrent affective disorder. Psychopharmacol. Bull. 16:47-49, 1980.

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Goodwin, F.K., Prange, A.J., Jr., Post, R.M., Muscettola, G., and Lipton, M.A.: L-triiodothironine converts tricyclic antidepressants non-responders to responders. Am. J. Psychiatry, in press.

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Zis, A.P., and Goodwin, F.K.: The amine hypothesis. In Paykel, E. (Ed.) Handbook of Affective Disorders. Churchill Livingstone Press, London, in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00450-07 CP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biological Rhythms in Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas Wehr	Chief, Clinical Res. Unit	CP/NIMH
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	Alfred Lewy	Staff Psychiatrist	NEI
	Philip Gold	Chief, Unit on Neuroendocrinology	CP/NIMH
	Anna Wirz-Justice	Visiting Scientist	CP/NIMH
	Norman Rosenthal	Staff Psychiatrist	CP/NIMH
	Gerard Groos	Assist. Professor	Univ. Leyden
	Richard Kronauer	Professor	Harvard
	Theodore Colburn	Chief	RSB/NIMH
	Bruce Smith	Biomedical Engineer	RSB/NIMH
	William Vaughan	Computer Programmer	RSB/NIMH
	Lawrence Tamarkin	Staff Fellow	IRP/NICHD

COOPERATING UNITS (if any) Research Services Branch, NIMH; Unit on Sleep Studies, Biological Psychiatry Branch, NIMH; Albert Einstein Medical School, New York; Harvard University, Cambridge; University of Leyden, Netherlands; Intramural Research Program, NICHD.

LAB/BRANCH
Clinical Psychobiology Branch

SECTION

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NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.1	2.6	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Basic research in biological rhythms can be related to affective illness with respect to (1) the inherent cyclicity of the illness (itself a type of biological rhythm) and (2) the involvement of disturbed circadian rhythms (24-hour cycles) in its pathophysiology. This project is designed to explore these connections by interrelating behavioral, physiological and biochemical changes observed (1) through the cycle of the illness, and (2) through the course of the 24-hour day. Results to date indicate (1) in depression the timing of circadian rhythms is abnormally early (phase-advanced) relative to the day-night cycle; (2) normals' sleep, temperature patterns and neuroendocrine function can be made to resemble that of depressives by shifting their sleep period 6 hours later than usual; (3) some depressives' circadian rhythm phase-advance and mood disturbances can be temporarily corrected by shifting their sleep period 6 hours earlier than usual; (4) animal studies in our laboratory indicate that the two major classes of drugs which are used to treat depression may act by slowing the intrinsic rhythm of circadian pacemakers causing a corrective delay in depressives' abnormally advanced circadian rhythms, (5) in mania the 24-hour sleep-wake cycle lengthens dramatically to 48 hours and escapes from its normal 1:1 mode of coupling to the day-night cycle; (6) manic depressives' circadian rhythm disturbances may be partly related to their abnormal sensitivity to light, a stimulus that controls the timing of circadian rhythms.

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

J. Christian Gillin	Chief, Unit on Sleep Studies	BP/NIMH
Marion Kafka	Research Psychologist	BP/NIMH
Elliot Weitzman	Chairman, Dept of Neurology	Einstein Med Sch.

Project Description:

Epidemiological evidence has emphasized the essential cyclicity of both unipolar and bipolar affective illness. Since manic-depressive illness is a periodic illness, a study of the role of biological rhythms in its pathophysiology and pathogenesis is important. This project involves longitudinal studies of behavioral, physiological, and biochemical variables in inpatients and outpatients who have relatively frequent mood cycles. Focus is on both circadian (i.e., 24-hour) and longer rhythms, requiring both intensive data collection (multiple sampling within the same day) and longitudinal data collection over time (in several patients, we have obtained daily measures for many months).

Behavioral parameters studied involve both nurses' ratings and self-ratings, as well as motor activity monitoring using a novel nontelemetric solid state, computer-based device developed at NIH. Physiological parameters include EEG sleep studies, temperature, biochemical variables including urinary 3-methoxy-4-hydroxyphenylglycol (MHPG), the urinary metabolite thought best to reflect brain norepinephrine metabolism, melatonin, cortisol, thyroid stimulating hormone (TSH), growth hormone (GH), prolactin (PL), and (less frequently) CSF amine metabolites. These data are evaluated by time series analyses and other methods in order to characterize circadian rhythms.

We can describe the findings in these studies under several headings:

1. Circadian rhythms are 24-hour cycles of biological functions which are driven by neural pacemaker cells in the hypothalamus. These rhythms have characteristic timing and waveforms which are genetically determined and homeostatically conserved. For example, body temperature rises each day to an evening peak, then declines to a late night low. We have studied the timing or phase-position of circadian rhythms in depressives compared with normals and compared with themselves in non-depressed states. Previously we found that the timing of a variety of circadian rhythms is abnormally early in depressives. This work has been extended and confirmed with additional data on REM sleep, rectal temperature, activity, and plasma melatonin all indicating a phase-advance.

We have continued to study bipolar patients longitudinally through one or more manic-depressive cycles. In these patients the timing of the circadian temperature rhythm became progressively earlier until the patient switched into depression; it then became progressively later until the patient switched out of depression. The range of these shifts was 5 to 12 hours. The finding of a maximal phase-advance at the switch into depression is consistent with our finding that circadian rhythms are phase-advanced in depressives compared with controls, a conclusion also consistent with our systematic review of the literature. Our interpretation that extensive sleep EEG findings in depression represent a phase-advance has stimulated considerable interest among sleep researchers as well as circadian physiologists. In patients in whom sleep EEG were studied longitudinally we continue to find parallel state-dependent changes in the timing of the temperature rhythm and REM sleep abnormalities.

For studies of temperature we have continued to use a self-contained, solid-state rectal temperature recorder which patients wear on their belt, and which monitors temperature continuously. Data from these instruments can be fed directly into a computer for subsequent plotting and analysis. A large scale cross-sectional study of circadian temperature rhythms and EEG-monitored sleep in depressed outpatients is underway. To date we have measured core body temperature in this manner in eleven depressed patients and ten normal subjects. Normal subjects' rectal temperature minimum occurred in the latter half of the sleep period. Rectal temperature minima occurred in the first half of the sleep period in those patients (N = 6) who also exhibited the typical pattern of REM sleep distribution seen in endogenous depression. Patients whose temperature minima fell in the latter half of the sleep period had normal REM sleep patterns. These results lend further support to the hypothesis that REM sleep abnormalities in endogenous depression are partly the result from a phase-advance of the circadian pacemaker that controls core temperature.

2. We have hypothesized that the phase-advance of circadian rhythms in relationship to sleep is a critical factor in establishing and maintaining the depressive state. Depriving a patient of sleep during the entire night often induces a dramatic, but short-lived, remission of the depression, suggesting that disrupting the abnormal relationship of sleep to circadian rhythms may be therapeutic. The early timing of circadian rhythms in relationship to sleep could be corrected if depressives' sleep schedule were shifted correspondingly earlier. We had previously studied one patient, in whom shifting the onset of sleep from 11 p.m. to 5 p.m. produced a remission which lasted two weeks. Following relapse, the patient's sleep was again shifted 6 hours earlier, producing a second remission. Recently this experiment was repeated with a depressed manic-depressive patient. EEG and rectal temperature were measured two weeks before and one week during the shifted sleep schedule. On the normal 11 p.m. to 7 a.m. sleep schedule temperature minima and REM sleep occurred abnormally early, near the beginning of the sleep period. When the sleep period was shifted 6 hours earlier (5 p.m. to 1 a.m.) temperature minima and REM sleep occurred near the end of the sleep period -- a normal pattern. On the second day of the shifted sleep schedule the patient switched out of depression. When the patient eventually returned to a habitual 11 p.m. to 7 a.m. sleep schedule the patient relapsed again into depression. The results of these preliminary experiments suggest that the circadian rhythm abnormalities in depression are not merely epiphenomena but play a causal role in the illness.

3. The depressive circadian rhythm disturbance can be simulated in normal subjects by delaying the timing of their sleep period later than usual e.g., from 11 p.m. - 7 a.m. to 7 a.m. - 3 p.m. By shifting sleep later relative to other circadian rhythms, the timing of other circadian rhythms is earlier relative to sleep, as in depressives. In our ongoing study, normal subjects' sleep is delayed 4, 8, and then 12 hours. After each shift, sleep EEG, temperature pattern, urinary free cortisol excretion the TSH response to TRH infusion are measured. So far six subjects have completed the entire experiment. To date, only sleep EEG and temperature data have been analyzed. In contrast to one previous study our results indicate that REM sleep density, which is high in depression, may also be related to circadian rhythm phases.

4. Earlier work in the Branch had demonstrated that chronic treatment with either imipramine (a tricyclic anditdepressant) or clorgyline (an MAO inhibitor) produced delays in the timing of the rest activity cycle in hamsters and in the phase position of the circadian rhythm in a number of specific binding sites ("receptors") for a variety of neurotransmitters. During the last year related work has been undertaken in collaboration with Lawrence Tamarkin of the NICHD. It was found that clorgyline (a type A monoamine oxidase inhibitor) and pargyline (a type B MAOI) delays the timing of the activity-rest cycle by several hours in a dose-dependent manner in the hamster. This result confirms our earlier findings that clinically active antidepressants delay the timing of circadian rhythms. In another experiment we found that clorgyline blocks the effects of light on melatonin secretion by the pineal gland. Thus, the drug may alter the timing of circadian rhythms by changing the sensitivity of the organism to light, an important environmental time cue that controls phases of circadian rhythms.

5. Another set of experiments addressing this question was done in collaboration with Gerard Groos of the University of Leyden (Dr. Groos will be joining our Branch as a Visiting Scientist in November, 1981). Clorgyline was injected directly into the suprachiasmatic nucleus (the circadian pacemaker in the hypothalamus) of rats whose circadian rhythms were free-running in isolation from external time cues. The drug temporarily abolished the circadian rhythm; when the rhythm reappeared in several animals it was considerably slower than before. Thus, the slowing effect of the drug may depend on a direct action on the pacemaker that drives circadian rhythms. Future pharmacological investigations of the suprachiasmatic nucleus are planned in our laboratory under Dr. Groos' direction.

All of these results are consistent with the hypothesis that antidepressant drugs act by slowing the circadian pacemaker and delaying the abnormally advanced circadian rhythms in depressed patients. Two major methodological questions are under further exploration. First, are these effects specific for clinically active antidepressants? Clinically inactive isomers must thus be tested in similar experiments. Second, to what extent did the effects of maintaining the animals on a light-dark schedule confound the observed effects of drugs on neurotransmitter binding rhythms? Future studies must be carried out in such a way that all measurements before and after drug-treatment are done in darkness.

6. Definition of phase, amplitude, and levels of circadian rhythms requires techniques and instruments which permit around the clock, continuous measurement of relevant variables. Longitudinal studies in patients require that these measurements be carried out day after day for months at a time. Fortunately, the development of a small solid state instrument by Theodore Colburn and his associates at the Research Services Branch has made it possible to obtain continuous longitudinal measurements of one variable: motor activity. We have studied the behavior activity-rest (sleep-wake) cycle for many months in 15 rapidly cycling manic-depressive patients. Our principal finding continues to be that the sleep-wake cycle lengthens from its usual 24-hour period to 48 hours at the onset of mania. Sometimes three or more double length sleep-wake cycles

were observed to occur in succession. We infer that the driving oscillator of the sleep-wake cycle markedly slowed its rate of oscillation and escaped from the primary (1:1) to the secondary (1:2) mode of entrainment to the day-night cycle. Similar lengthening of the period of the sleep-wake cycle and shift into and out of the secondary coupling mode also occurs in normal subjects during free-running in isolation experiments. In these normals, the sleep-wake cycle lengthens, escapes from 1:1 coupling with the free-running 25-hour cycles of the circadian temperature rhythm and recouples in a 1:2 mode thus, the resulting 50-hour sleep-wake cycle locks onto every other 25-hour temperature cycle. When this occurs, normal subjects may remain wake for 30 hours or more with no subjective need for sleep. Manics, too, may stay awake the entire night with no perceived need for sleep.

There are several posible causes of these abnormally long sleep-wake cycles during mania. One possibility is that the intrinsic rhythm of a driving oscillator of the sleep-wake cycle is abnormally slow and thus cannot consistently keep up with the 24-hour rhythm of the day-night cycle, resulting in periodic 48 hour sleep-wake cycles when the coupling shifts from 1:1 to 1:2.

With regard to this possibility, our animal studies indicate that antidepressant drugs may slow the intrinsic rhythm of oscillators which drive circadian rhythms. This slowing could be expected to precipitate abnormal slowing of the sleep-wake oscillator, and might slow the oscillator sufficiently to induce the 48 hour sleep-wake cycle seen at the onset of mania.

It is possible that some of the circadian rhythm abnormalities in manic-depressives are related to an abnormal sensitivity to environmental stimuli, such as light, which function as time cues for the circadian pacemaker. For example, the abnormally advanced phases of circadian rhythms in patients could result from supersensitivity to light. We previously determined that in normal subjects melatonin secretion by the pineal gland could be suppressed by approximately 1500 to 2500 lux light (this effect is mediated by some of the same neural pathways that synchronize the circadian pacemaker with light-dark cycles). During the past year we found that melatonin secretion in ten manic-depressives could be suppressed by 500 lux, a light intensity much lower than that required for normal subjects. These results indirectly support the hypothesis that some of the patients circadian rhythm abnormalities are related to their sensitivity to external time cues.

Our studies of patients living in a normal environment have raised several questions that can only be answered by studying patients whose circadian rhythms are free-running while they are isolated from all external time cues. For example, do phase-advanced circadian rhythms in depressives result from an overly fast intrinsic rhythm of the circadian pacemaker? We are attempting to explore these and other questions by establishing conditions suitable for free-running experiments on the 4-West research unit.

7. In population statisitics and in individual cases depression and mania show seasonal patterns of occurrence. Seasonal rhythms in animals depend on the ability of a circadian clock to measure changes in the daily photoperiod (interval between dawn and dusk). Thus, it is possible that seasonally occurring depressions are triggered by changes in the photoperiod. Under the direction of Dr. Norman Rosenthal we are currently exploring this possibility in two ways. First we have initiated a large scale survey of patients with seasonal patterns of depression. To date, over 400 patients have been identified in the Washington, D.C. area. Through questionnaires and interviews, the phenomenology of seasonal depressions will be documented. In addition, a longitudinal prospective study of changes in biological and behavioral variables through the course of the year will be undertaken, including behavioral ratings, sleep EEG studies, motor activity recordings, temperature measurements, and selected endocrine tests. In one patient with a 20 year history of winter depressions, we were able to abort a depressive episode in December by exposing the patient to artificial spring lighting conditions. In a small number of selected patients we intend to explore whether similar manipulations of the photoperiod can indeed prevent winter depressions. This approach might offer a non-pharmacological treatment modality for patients who are refractory to drugs.

Awards and Honors:

Drs. Wehr and Wirz-Justice were awarded third prize of the Anna-Monika Foundation award for original research in depression for the studies of circadian rhythms and affective disorders carried out in the Clinical Psychobiology Branch.

Publications:

Wehr, T.A. and Goodwin, F.K.: Circadian rhythm desynchronization as a basis for manic depressive cycles. Psychopharmacol. Bull. 16:19-20, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01851-04 CP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Melatonin Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Thomas Wehr	Chief, Clinical Research Unit	CP/NIMH
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	Irwin J. Kopin	Chief	LCS/NIMH
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	Ernest Ballintine	Chief	CB/NEI

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Clinical Psychobiology Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.2	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of the pineal gland and its hormone, melatonin, has been difficult to define in humans, because the circulating levels of melatonin have been too low to measure. In collaboration with Dr. S. Markey of the LCS, we have developed a highly sensitive and specific gas chromatographic-mass spectrometric (GC-MS) assay which requires less than 1 ml of human plasma, CSF, or urine. We are currently investigating: (1) the circadian pattern of secretion of melatonin in normal individuals and its lateration in a variety of disease states (such as depression, mania, and blindness); (2) sensitivity of patients and normal subjects to light suppression of nocturnal melatonin secretion; (3) the effect of drugs on light suppression of melatonin secretion. We have found that manic-depressive patients are abnormally sensitive to light; that clorgyline, a monoamine oxidase type A inhibitor that is used to treated manic-depressives desensitizes hamsters' melatonin suppression by light; and that blind subjects have markedly abnormal timing of the circadian rhythm in melatonin secretion, in some cases because the rhythm is no longer entrained to the day-night cycle, but is free-running according to its own 25-hour rhythm. This last result underlines the importance of light and sensitivity to light in the normal homeostasis of circadian rhythm phases (phases that are disordered in manic-depressive illness).

Project Description:

Objectives: The pineal gland is of considerable interest not only because its basic role in endocrine function in man is unknown, but also because it provides an in vivo marker for the biological clock regulating the activity-rest cycle (see below), as well as being a measure of the activity of a well-characterized beta-adrenergic receptor. In man, plasma levels of melatonin are extremely low, on the order of 1-2 picogram/ml during the day, not quantifiable by previous techniques. Dr. Lewy has developed a gas chromatographic-mass spectrometric (GC-MS) method for the measurement of melatonin in collaboration with Dr. Sanford P. Markey of the LCS. This technique has the requisite specificity and accuracy to measure melatonin in humans in quantities as little as 1 cc of plasma. In addition to affective illness, other diseases of interest include those involving the thyroid and adrenal, precocious and delayed onset of puberty, infertility, hypertension, malignancy, including cancer of the breast and ovary, and blindness.

The neurotransmitter norepinephrine is the normal physiologic stimulant of the pineal gland's secretion of melatonin. Attention has been focused on this neurotransmitter in conjunction with affective disorders and other psychiatric diseases. We are studying the effects of various psychotropic medications, including tricyclic antidepressants, lithium, neuroleptics, and MAO inhibitors, both acutely and chronically. Many of these medications have been thought to induce changes in β-adrenergic receptors. It is now possible to document in vivo these changes in humans.

Methods: Although radioimmunoassays (RIAs) and bioassays were used for measurement of human plasma melatonin, problems with insufficient sensitivity, specificity or efficiency have severely limited their use for routine analysis. Whereas theoretically GC-MS is the most specific of these techniques, attempts to apply it to melatonin analysis have also failed because of insufficient sensitivity. Use of a new technique, negative chemical ionization, and improved chromatographic and extraction procedures, led to a GC-MS method that has the requisite sensitivity, specificity and efficiency for the routine analysis of melatonin in human plasma, CSF, and urine. Less than 1 ml of sample is required, thus, permitting wide flexibility of experimental design and repeated and frequent measurements. Daytime concentrations as low as 1-2 picogram/ml and night-time values as high as 100 picograms/ml can be measured, yielding a 20-50 fold range. The other available assays (RIAs and bioassays) generally report higher values and a lower range, probably because of their lack of specificity.

Major Findings: We previously determined that human melatonin is secreted only at night, as in other species. We also demonstrated that human nocturnal melatonin secretion could be suppressed by light; however, humans are relatively insensitive to light, compared with other species. Humans require 1500 to 2500 lux to completely suppress the hormone. During the past year we have studied manic-depressives' response to light. In all 12 patients studied, we found that low levels of artificial light (500 lux) were enough to suppress nocturnal melatonin secretion. Thus, patients appear to be hypersensitive to light. This results is consistent with anecdotal reports that patients are

subjectively sensitive to light (many wear sunglasses indoors, for example). Their unusual sensitivity to light may partly explain circadian rhythm phase disorders een in these patients, since light is an important environmental stimulus that synchronizes circadian rhythms to the day-night cycle.

In the hamster, we found that clorgyline, a monoamine oxidase type A inhibitor used to treat manic-depressive patients, blocks light suppression of melatonin secretion. Currently we are studying the effect of the drug on light suppression of melatonin secretion in patients. The results of the animal study suggest that the drug might make patients less sensitive to light--i.e., more nearly normal in this respect.

Because of our interest in the role of light as an external time cue that synchronizes circadian rhythms to the day-night cycle, we have also studied patterns of melatonin secretion in blind persons in collaboration with Drs. Newsome and Ballintine of the National Eye Institute. Our first study revealed that the phases of the melatonin circadian rhythm were markedly abnormal in blind persons. Some of them secreted melatonin only in the daytime, instead of the normal nighttime pattern. In a subsequent longitudinal study we found that a subgroup of blind persons actually had free-running melatonin circadian rhythms; that is, the rhythm was no longer entrained to the 24-hour day-night cycle, but followed its own nearly 25-hour intrinsic rhythm. These results confirm that light plays an important role in the synchronization of human circadian rhythms to the 24-hour day-night cycle, and lends some support to the hypothesis that manic-depressives circadian rhythm disturbances may be partly related to their abnormal sensitivity to light.

Publications:

Lewy, A.J., Wehr, T.A., Goodwin, F.K., Newsome, D.A., and Markey, S.P.: Light suppresses melatonin secretion in humans. Science 210:1267-1269, 1980.

Lewy, A.J., Wehr, T.A., Goodwin, F.K., Newsome, D.A., and Rosenthal, N.E.: Manic-depressives may be supersensitive to light. Lancet, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00447-12 CP

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Amine Neurotransmitters and Metabolites in Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Frederick Goodwin	Chief	CP/NIMH
OTHER:	Rex Cowdry	Chief, Outpatient Unit	CP/NIMH
	Markku Linnoila	Staff Psychiatrist	CP/NIMH
	William Potter	Chief, Unit on Clin. Psychopharm.	CP/NIMH
	Thomas Wehr	Chief, Clinical Research Unit	CP/NIMH
	Paul Marangos	Chief, Unit on Neurochemistry	CP/NIMH
	Philip Gold	Chief, Unit on Neuroendocrinlogy	CP/NIMH
	Robert Post	Chief, Clinical Research Unit	BP/NIMH
	Edna Gordon	Chief, Unit on Clinical Biochemistry	LCS/NIMH
	Daniel van Kammen	Chief, Unit on Neuropsychopharm.	BP/NIMH
	David Rubinow	Staff Psychiatrist	BP/NIMH
	Michael Ebert	Chief, Section on Exp. Therap.	LCS/NIMH
	James Ballenger	Guest Worker	BP/NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH; Biological Psychiatry Branch, NIMH; Clinical Neuropharmacology Branch, NIMH; University of Naples, Naples, Italy.

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Clinical Psychobiology Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.1	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Amine neurotransmitters and their metabolites are studied in blood, cerebrospinal fluid (CSF), and urine to assess neurochemical alterations which may be associated with clinical diagnoses, clinical states, particular behavioral or psychopathological dimensions, or treatment with drugs. Because of both methodological advances in assays for low concentrations of metabolites and problems associated with the probenecid technique, CSF studies have focused increasingly on baseline (non-probenecid) lumbar punctures. A high pressure liquid chromatographic assay for monoamine neurotransmitters and their metabolites in blood, wine, and CSF was developed. Application of this assay to urinary measurements of norepinephrine and its metabolites provides an important new interpretation of the meaning of urinary 3-methoxy-4-hydroxy-phenylglycol levels. Two further studies confirm previous reports of an association between low CSF 5-hydroxyindoleacetic acid and several measures of impulse dyscontrol. Drug response prediction studies based on pre-treatment metabolite levels continue.

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

David Jimerson	Staff Psychiatrist	LCS/NIMH
Dennis Murphy	Chief	CN/NIMH
David Pickar	Chief, Unit on Studies of Drug Abuse	BP/NIMH
Giovanni Muscettola	Associate Professor	U. of Naples

Project Description:

The purpose of this project is the direct assessment of the functional state of brain neurotransmitter amines in patients and normal individuals by assay of levels of the amines and their major metabolites in plasma, in urine, and in the cerebrospinal fluid (CSF). The metabolites under study are 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of serotonin; homovanillic acid (HVA), the major metabolite of dopamine; and 3-methoxy-4-hydroxyphenylglycol (MHPG) and 3-methoxy-4-hydroxyphenylacetic acid (VMA), the major metabolites of norepinephrine.

The Chief, CPB, serves as the coordinator for CSF studies in the Division; the Unit on Neurochemistry, CPB provides sample processing and assays (Dr. Markku Linnoila in collaboration with Edna Gordon and Michael Ebert of the LCS); this Branch also provides central data storage and analysis for CSF results. The major collaborating groups for the CSF studies are the Sections on Psychobiology (Dr. Post) and Neuropharmacology (Dr. van Kammen) of the Biological Psychiatry Branch and the Clinical Neuropharmacology Branch (Dr. Murphy). Studies from the Clinical Psychobiology Branch are reported in detail here. Studies from collaborating groups are reported in the annual reports of the groups involved.

Methodological Issues

Cerebrospinal fluid studies have previously involved both "baseline" lumbar punctures (LP's) and LP's following the administration of probenecid, an organic acid which competitively inhibits the active transport of acid metabolites from CSF. However, completed studies suggest that there are major sources of variance attributable to the probenecid methodology: the incidence of nausea and vomiting is related to CSF probenecid levels and is independent of dose; weight changes during hospitalization affect CSF probenecid levels; CSF metabolite accumulation is significantly correlated with CSF probenecid levels, but the strength of the correlation varies markedly among diagnostic groups; and medications such as lithium affect the CSF metabolite-probenecid correlation.

Previously, one of the prime justifications for the probenecid technique was the high coefficient of variation of metabolite levels in baseline studies when fluorometric assays were used. In contrast, more recently available gas chromatographic-mass spectrometric (GC-MS) assays, performed by Edna Gordon, Michael Ebert, and David Jimerson, produce relatively sensitive, specific, and reproducible results even at the low metabolite concentrations found in non-probenecid CSF studies. Because of this, we have increasingly focused on CSF studies without the probenecid methodology.

However, GC-MS assays suffer from several drawbacks, including the need for derivitization steps and the relative scarcity of time on the mass spectrometer. Because of these drawbacks, Dr. Markku Linnoila has done extensive work on the development of a high pressure liquid chromatographic (HPLC) method for assaying CSF metabolites. Values obtained using Dr. Linnoila's HPLC method agree closely with those obtained in parallel GC-MS assays, suggesting that the HPLC will provide an accurate and available methodology for routine analysis of clinical specimens. Dr. Linnoila has also developed HPLC assays for the neurotransmitters themselves, which, when fully validated, will enable us to measure both neurotransmitters and their metabolites in a single sample using a single

methodology, rather than relying on a series of measurements by different collaborators in different laboratories utilizing different methodologies.

It should be noted that the availability of essentially simultaneous measurements of monoamine neurotransmitters and their metabolites using a single methodology opens a new realm of inference, including the study of complex interrelationships between neurotransmitter release, reuptake, and degradation in single neurotransmitter systems and the interrelationships among catecholamine and indoleamine neurotransmitter systems.

Clinical Studies of Monoamine Neurotransmitters and Their Metabolites

In collaboration with other NIMH units, we have continued the exploration of CSF amine metabolites within and between various clinical states and diagnostic groups (affective illness, schizophrenia, schizo-affective disorder, and other disorders such as anorexia nervosa). Pre-treatment levels of monoamine neurotransmitters and metabolites are also measured as possible predictors of response to treatments with drugs such as desmethylimipramine, zimelidine, and tranylcypromine. In addition, drug trials are generally designed with a cerebrospinal fluid study before and during treatment, to document biochemical effects of the drugs which may be associated with treatment in general, or with therapeutic response specifically. During the past year, clinical samples have been collected but not assayed pending the final validation of the HPLC methodology. With the final availability of the HPLC assay, we expect to complete a broad series of studies during the coming year.

The primary new clinical finding is an extension of previous studies associating serotonin metabolism with impulsive or violent behavior. Previous studies from Scandinavia suggest that low levels of CSF 5-HIAA may be associated with violent suicide attempts in both depressed and non-depressed subjects. This finding corresponds to a previous finding reported by our Branch in collaboration with the National Naval Medical Center, suggesting that, among young males with character disorders, low CSF 5-HIAA is relatively strongly associated with a lifetime history of recurrent violent and often impulsive acts. Two new studies completed this year give further weight to this association and extend the association further across traditional diagnostic boundaries. Another study performed by Dr. Lavonne Brown in collaboration with our Branch and the National Naval Medical Center suggests that low CSF levels of 5-HIAA are significantly correlated with high scores on the Pd scale of the MMPI, and are nearly significantly correlated with a lifetime history of violent acts, when studied in a group of individuals with borderline personality disorder. A retrospective chart review of patients with major depressive disorder studied on our inpatient unit over the past eight years suggests that there is a significant correlation between low levels of CSF 5-HIAA following probenecid administration and the occurrence of significant episodes of behavioral dyscontrol at some point during the patient's lifetime. This association was nearly significant both for overt attempts at suicide and for overt acts of violence, and becomes quite significant when suicide and violence are considered as alternative forms of behavioral dyscontrol. These findings suggest that certain biochemical abnormalities may have behavioral significance which cuts across traditional diagnostic boundaries, and further suggests that some biochemical abnormalities may represent a trait marker or an enduring predisposition to certain forms of behavior.

Our urinary studies of amine metabolites have focused exclusively on MHPG in previous years. Methodological and theoretical issues in the study of urinary MHPG as an indicator of central noradrenergic function have been reviewed in three publications from our Branch which appeared during the past year. Currently, new methods have enabled us to expand our studies of the noradrenergic system to include measurement of norepinephrine and its metabolites in the same samples of urine. In 12 patients, we find correlations of 0.83 to 0.92 among urinary excretion rates of NE, MHPG, normetanephrine, and vanillylmandelic acid; and correlations of 0.91 to 0.99 when any one of the metabolites is correlated with the total excretion of all four substances taken together (a measure of total body NE turnover at steady-state). The finding that urinary MHPG excretion rates in particular correlate highly with total body norepinephrine turnover suggests that urinary MHPG cannot be used specifically as an indicator of turnover rates in the central nervous system (CNS), except insofar as CNS turnover rates themselves correlate highly with total body turnover rates. If validated in a larger sample, these findings would have widespread implications regarding the clinical and research utility of measurements of urinary MHPG.

Further Directions

The availability of a brief inpatient evaluation program will enable us to examine monoamine metabolites in a broader spectrum of depressed patients. Of particular interest is whether patients with non-endogenous depressive syndromes have high urinary MHPG (as suggested by Schildkraut) and whether individuals with a history of difficulties with control of affect and impulses demonstrate low CSF 5-HIAA. Monoamine neurotransmitters and metabolites will be examined in connection with putative biological markers such as blunted TSH response to TRH and failure of exogenous dexamethasone to suppress cortisol. Such inter-correlations may give further insight into monoaminergic regulation of hypothalamic pituitary axes. Finally, the HPLC assays will enable us to make more specific statements about the biochemical effects of specific drugs, including desmethylimipramine, zimelidine, tranylcypromine, and "low dose" clorgyline.

Publications:

Cowdry, R.W., and Goodwin, F.K.: Biological and Physiological Predictors of Response. In van Praag, Rafaelson, Lader, and Sachar (Eds.): Handbook of Biological Psychiatry. New York, Marcel Dekker, Vol. VI, 1981, pp. 263-307.

Goodwin, F.K. and Potter, W.Z.: Noradrenergic Function in Affective Illness. In Saletu, B. (Ed.): Proceedings of the 11th CINP Congress. New York, Pergamon Press, 1979, pp. 127-137.

Muscettola, G., Potter, W.Z., Gordon, E.K., and Goodwin, F.K.: Methodological issues in the measurement of urinary MHPG. Psychiat. Res. 4: 267-276, 1981.

Potter, W.Z., Muscettola, G., and Goodwin, F.K.: Sources of Variance in Clinical Studies of MHPG. In Maas, J.W. (Ed.): MHPG and Psychopathology. New York, Academic Press. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01850-04 CP

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of Antidepressants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William Potter	Chief, Unit on Clinical Psychopharmacology	CP/NIMH
		Coordinator for Clinical Pharmacology	P-T/NIGMS
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	Markku Linnoila	Staff Psychiatrist	CP/NIMH
	Richard Ross	Staff Fellow	P-T/NIGMS
	Matthew Rudorfer	Staff Fellow	CP/NIMH
	Thomas Wehr	Chief, Clinical Research Unit	CP/NIMH
	Isamu Kitanaka	Visiting Fellow	CP/NIMH
	Mika Scheinin	Visiting Fellow	CP/NIMH
	Wen-Ho Chang	Visiting Fellow	CP/NIMH
	Elizabeth Lane	Visiting Fellow	P-T/NIGMS
	Anthony Zavadil	Guest Worker	LCS/NIMH
	Sanford Markey	Chief, Pharm. Appl. of Mass Spec.	LCS/NIMH

COOPERATING UNITS (if any) Pharmacology-Toxicology Program, NIGMS; Laboratory of Clinical Science, NIMH; Unit on Pharmacological Application of Mass Spectrometry, LCS/NIMH; Division of Special Mental Health Research, NIMH; Department of Clinical Pharmacology, Karolinska Institut, Sweden.

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.7	2.9	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on the clinical pharmacology of biochemically specific antidepressants produce both therapeutic advances and increased knowledge concerning the biological basis of affective illness. Further investigations of pharmacokinetic variance have shown that renal clearance is a mjaor determinant in the elimination of active hydroxy metabolites of tricyclic antidepressants and may explain altered drug response in the elderly. In vitro and in vivo animal and human studies are elucidating the extent to which active hydroxy metabolites are important and the mechanisms which determine their concentrations. Identifying the pharmacodynamics (effect produced by a known concentration) of selected antidepressants has advanced in several areas: (1) demonstration of increased functional norepinephrine combined with decreased total production following desipramine; (2) finding a possible dopaminergic effect of desipramine indexed by increased prolactin; (3) questioning whether a serotonergic effect of zimelidine accounts for increased vasopressin, not found after other drugs; (4) discovering an unexpected common reduction of total NE production after both drugs; (5) using sufficiently low doses of clorgyline to selectively inhibit MAO-A in man has provided a powerful new treatment for previously unresponsive bipolar rapid cyclers. This latter finding may provide a therapeutic break through.

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

Irwin J. Kopin	Chief	LCS/NIMH
Richard J. Wyatt	Chief	DSMHR/NIMH
Neal Cutler	Staff Psychiatrist	DSMHR/NIMH
Farouk Karoum	Neurochemist	DSMHR/NIMH
Folke Sjoqvist	Chairman, Dept. of Clin. Pharmaccology	Karolinska
John Nurnberger	Staff Psychiatrist	AP/NIMH
Elliot Gershon	Chief, Secton on Psychogenetics	AP/NIMH

Project Description:

Continued expansion of studies on the clinical and pharmacological effects of antidepressant drugs has been possible as well as completion of certain former projects. Until his departure in June, 1981 Dr. Isamu Kitanaka, a Fogarty Visiting Fellow, was responsible for pharmacokinetic studies of desipramine in man and animals. Markku Linnoila, M.D., Ph.D. on an IPA from Duke University has made an outstanding contribution in terms of high performance liquid chromatograph (HPLC) method development for assessing biochemical effects of antidepressants as well as initiating new clinical studies (see below). Since January, 1981, Dr. Linnoila has been assisted by Dr. Mika Scheinin, a Fogarty Visiting Fellow from Finland. Richard Ross, M.D., Ph.D., a PRAT fellow, has completed an important study on the effects of a biochemically specific antidepressant in man. As of July, 1981 three additional investigators have become involved in ongoing pharmacokinetic and pharmacodynamic studies: Dr. Wen-Ho Chang, the first investigator from the Peoples Republic of China to work with the NIMH as part of an official joint US-PRC Health agreement signed last fall; Dr. Matthew Rudorfer, a psychiatrist selected by the PRAT program to study clinical pharmacology, and Dr. Elizabeth Lane, a Ph.D. pharmacokineticist, selected as a Fogarty PRAT clinical pharmacologist. In addition collaborations between our unit, other institutes and even other centers have been maintained and expanded.

Assay Development: The application of an established gas chromatographic-mass spectrometric (GC-MS) assay for tissue concentrations of tricyclic antidepressants (TCAs) has continued to be used for specialized problems. In the main, however, our established and validated HPLC assay for TCAs and their hydroxylated metabolites has been used. We have modified it to permit drug analysis in urine as well as plasma from both patients and animals.

We have developed two liquid chromatographic methods for the measurement of MHPG, HVA and 5HIAA, and 5HT in biological samples. These assays are distinguished from existing methods by mobile phases which consist of organic amine bases and and by unique internal standards 6-fluoro HVA and 6-fluoro 5HT, which were synthesized on the NIH campus at our request. These developments yield a greater accuracy and shorter chromatography times than any existing liquid chromatogarphic assay systems for these substances. In addition, we have deployed a liquid chromatographic assay for the neurotransmitters dopamine and norepinephrine. This combination of assays allows us to quantitate these substances of interest in a 3.5 ml aliquot of human cerebrospinal fluid, or plasma or urine and in very small pieces of brain tissue of experimental animals.

Pharmacokinetic Studies:

1. Completion of a study using single dose pharmacokinetic predictions of therapeutic drug doses (Z01 MH 01850-01 CP) in subjects of all ages yielded a safe and useful tool especially for research in volunteers and the elderly. Our studies of the aged done in collaboration with Dr. Neal Cutler show clearly that the pharmacokinetics of parent desipramine is not altered in the elderly thereby clarifying a disputed point.

2. As part of our studies in (1.) we observed that the concentration of the active hydroxy metabolite of desipramine, initially identified by our earlier investigations (Z01 MH 01850-02 CP), is elevated in the elderly. Moreover this elevation does not appear to be due to altered drug metabolism but to decreased renal clearance of the metabolite -- perhaps a "normal" concomitant of aging.

3. Prospective studies by Dr. Elizabeth Lane employing recently developed pharmacokinetic models have just begun to clarify the relative contributions of tricyclic metabolism vs. renal elimination in determing effective drug concentration. Findings will be highly relevant to antidepressant drug use in the elderly as well as in any patients with altered hepatic or renal function.

4. Prior to his departure Dr. Kitanaka was able to demonstrate that previous studies of desipramines' pharmacokinetics in the rat arrived at highly erroneous conclusions (because of method limitations). These findings have important implications for the interpretation of desipramine's (and other TCAs') effects in rats which are currently being used to support hypotheses concerning the mode of action of antidepressants.

5. As part of a study performed while Dr. Potter was an invited Visiting Scientist at the Department of Clinical Pharmacology of the, Karolinska Institut in Sweden last summer, a pharmacokinetic "mystery" has been clarified. Based on our experience with GC-MS assays of TCAs we were able to suggest an explanation for the failure to detect metabolite formation from a TCA following intramuscular administration. This was tested by Drs. Potter and Folke Sjoqvist (who briefly worked with us here) with the demonstration that TCA metabolism was not fundamentally altered by the intramuscular route. Moreover, it was shown, using human liver microsomes in vitro, that the major determinant of active drug elimination, hydroxylation, is similar for the major classes of antidepressants. Complimentary studies will be continued here at the NIMH as part of the renal clearance investigations (see 3. above).

6. As part of the general task of using pharmacokinetic approaches to controlling for active drug concentrations, comparisons of chemical (HPLC) and biologic (radioreceptor) assays have led to reservations concerning the latter. The chemical assays of, for instance, thioridazine and its active metabolites have been found to be internally consistent with steady-state assumptions while the radioreceptor assay produces anomalous results. We therefore emphasize the critical importance of percise identification of each active drug form.

Clinical and Biochemical Effects of Antidepressants:

1. A unique double-blind cross-over trial in which an experimental antidepressant (zimelidine), specific for serotonin uptake inhibition, is compared with an established antidepressant (desipramine) specific for norepinephrine uptake inhibition has been completed. The finding that zimelidine dose not precipitate mania or rapid cycles in bipolar depressed patients (as contrasted to desipramine) has provided a basis for an outpatient trial of zimelidine with or without lithium in bipolar patients. If zimelidine

proves to be an effective antidepressant in such patients a major therapeutic advantage will result. This study is being done in collaboration with Drs. Nurnberger and Gershon of the APB.

2. Biochemical analyses of urine from volunteers treated with desipramine in combination with our previous analyses of plasma permit clarifiation of the effects of a specific norepinephrine uptake inhibitor. Desipramine both decreases the deamination of norepinephrine (NE) and its total turnover. However, consistent with its effects on inhibiting uptake, plasma NE actually increases. This may explain the physiological effects described below.

3. It has been possible to show, for the first time, that both the acute and chronic effects of desipramine in volunteers are the same and are most consistent with increased adrenergic function. Parameters which support this are the pattern of pulse, blood pressure and EKG changes. Moreover, it is found that the drug, per se, does not cause significant orthostatic hypotension although it may aggravate it in individuals with a pre-existing problem. These findings have significant theoretical implications in that they do not support current notions of tolerance to TCAs.

4. Certain specific biochemical effects of desipramine vs. zimelidine have been confirmed and extended. These include the surprising finding of a stimulatory effect of desipramine on prolactin and the parallel finding that TCAs decrease HVA in the CSF and urine. These findings suggest that even a "pure" NE uptake inhibitor such as desipramine may have dopaminergic effects.

The unique effect of zimelidine as a stimulator of vasopressin has been given further support by findings in the periphery where the plasma vasopressin response to isotonic saline is augmented during treatment with the drug.

5. Despite separate biochemical effects zimelidine and desipramine produce a common effect of an overall body decrease in NE turnover as reflected by analysis of all metabolites in urine. This demonstrates the limitations of urinary metabolite measurements as a means of identifying drug action and suggests that numerous investigations are incorrectly interpreting the data available to them. Since urinary metabolites are being "marketed" as diagnostic and treatment tools our findings have particular meaning.

6. As part of our effort to find new ways of treating rapid-cycling manic depressive patients we have discovered that low dose clorgyline may offer a unique and safe treatment. Six treatment resistant (i.e. not responding to lithium $\pm$ standard antidepressants) patients have shown remarkable improvement. In two cases, complete remission of a previously intractable illness has been achieved. Biochemical studies indicate that even in low doses maximum MAO-A inhibition can be achieved without affecting platelet MAO or REM sleep. This discrimination of biochemical and neurophysiologic effects from clinical outcome and MAO-A inhibition has important theoretical and clinical implications.

7. As part of the biochemical assessment of clorgyline effects Dr. Linnoila has found that subjects with elevated phenylethylamine (PEA) respond

poorly to all treatments. Furthermore, drugs which increase PEA frequently if not inevitably lead to poor response in manic-depressive patients. Interestingly, low dose clorgyline does not increase PEA. These findings are consistent with the formerly postulated role of PEA as an "endogenous amphetamine" and may provide a means of selecting patients for treatment. Attempts to control at least the peripheral formation of PEA by administration of the decarboxylase inhibitor, carbidopa, are underway. Our preliminary experience suggests that control of PEA formation may provide a significant therapeutic advantage in patients whose PEA are elevated.

8. Efforts to understand the influence of the major classes of psychoactive drugs on psychomotor function have been initiated. These include evaluation of the tricyclic antidepressant zimelidine, alcohol, and benzodiazepines --- alone or in combination. The hypothesis is that drugs with truly "specific" effects will not show significant interactions among each other and hence will act on independent parameters of psychomotor function. It may therefore be possible to develop psychomotor testing as a means of evaluating hypotheses of receptor action and sensitivity for understanding the actions of drugs used in psychiatry.

Publications:

Potter, W.Z., Calil, H.M., Zavadil, A., Sutfin, T., Jusko, W.J., Rapaport, J. and Goodwin, F.K.: Steady-state concentrations of hydroxylated metabolites of tricyclic antidepressants in patients: Relationship to clinical effect. Psychopharmacol. Bull. 16 (2):32-34, 1980.

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Potter, W.Z., Calil, H.M., Extein, I., Muscettola, G. and Goodwin, F.K.: Cross-over study of zimelidine and desipramine in depression: Evidence for amine specificity. Psychopharmacol. Bull. 17:26-29, 1981.

Potter, W.Z., Calil, H.M., Extein, I., Gold, P.W., Wehr, T.A. and Goodwin, F.K.: Specific norepinephrine and serotonin uptake inhibitors in man: A cross-over study with pharmacokinetic, biochemical, neuroendocrine and behavioral parameters. Acta Psychiat. Scandinavica Suppl. 290, Vol. 63, pp. 152-165, 1981.

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Cutler, N.R., Zavadil, A.P., Eisdorfer, C., Ross, R.J. and Potter, W.Z.: Unexpected low concentrations of desipramine in elderly women. Am. J. Psychiatry, in press.

Linnoila, M., Rosenblatt, J.E., Jeste, D., Skinner, T., Potter, W.Z. and Wyatt, R.J.: Disparate serum thioridazine concentrations: Liquid chromatography vs. radioreceptor assay. Acta Pharmacologica et Toxicologica, in press.

Potter, W.Z. and Linnoila, M.: Tricyclic antidepressant concentrations: Clinical and research duplications. In Neurobiology of the Mood Disorders, R.M. Post and J.C. Ballenger (Eds.). Williams and Wilkins, Baltimore, Maryland, in press.

Kitanaka, I., Ross, R.J., Cutler, N.R., Zavadil, A.P. and Potter, W.Z.: Altered hydroxy desipramine concentrations in elderly depressed patients. Clin. Pharm. Therap., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00452-06 CP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neuroendocrine Studies of Major Psychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Thomas Wehr	Chief, Clinical Research Unit	CP/NIMH
	Leslie Becker	Clinical Associate	CP/NIMH
	Robert Post	Chief, Section of Psychobiology	BP/NIMH
	David Rubinow	Clinical Associate	BP/NIMH
	Daniel van Kammen	Chief, Section on Neuropsychopharm.	BP/NIMH
	Elliot Gershon	Chief, Section on Psychogenetics	BP/NIMH
	John Nurnberger	Senior Staff Fellow	BP/NIMH
	Walter Kaye	Clinical Associate	LCS/NIMH
	Michael Ebert	Chief, Section on Exp. Ther.	LCS/NIMH
	Seymour Reichlin	Chief, Division of Endocrinol.	Tufts Univ.
	Detlev Ganten	Prof. Dept. of Pharmacol.	U. of Heidelberg

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INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.5	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Current studies concentrate on comprehensive tests of hypothalamic-pituitary function and on the measurement and administration of specific behaviorally active peptides or peptide analogs. Major findings this year are: There is a significant reduction in cerebrospinal fluid (CSF) oxytocin in drug free bipolar patients in the depressed, manic, and recovered states. CSF vasopressin is significantly lower in drug free psychotic schizophrenic patients compared to controls. Des-amino-8-d-arginine vasopressin (DDAVP), a vasopressin analog, enhances specific components of information processing in patients with Alzheimer's disease analogous to its previously demonstrated effects in depressed patients and controls. In studies of AVP function in anorexia nervosa patients studied while chronically underweight and at intervals following weight gain, we note that not only do many show evidence of partial neurogenic diabetes insipidus, but many also show a previously undescribed syndrome of completely disrupted osmoregulation, which, when present, is consistently associated with marked abnormalities in CSF AVP. Zimelidine increases both CSF AVP and the plasma AVP response to hypertonic saline, compatible with findings in anorexia nervosa indicating common regulatory mechanisms for CSF and plasma AVP.

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

Gregory Brown	Chief, Dept. of Neurosciences	McMaster Univ.
Delbert Fisher	Professor of Medicine	UCLA
Lynn Lorioux	Chief,	DEB/NICHD
Richard Sherins	Senior Investigator	DEB/NICHD
George Chrousos	Senior Investigator	DEB/NICHD
Samuel Yen	Chief, Dept of Reproduction Endocrinology	UCSD
Gary Robertson	Professor of Medicine	Univ. of Chicago

Project Description:

Several aspects of the symptom complex of the functional psychoses, particularly affective illness, suggest hypothalamic dysfunction. Thus, patients with depression or mania often show disturbances in sleep, altered energy levels, changes in appetite and libido, diurnal variation in symptoms, alterations in the consolidation of memory traces, and changes in reproductive function such an amenorrhea. Interest in the hypothalamic-pituitary (HP) axis has been further stimulated by recent findings that the monoamine neurotransmitters modulate the synthesis and release of a number of hypothalamic peptides and pituitary hormones. Thus, examination of pituitary hormones in plasma can shed light on the functional activity of biogenic amine systems. Moreover, these peptides and hormones may have specific receptors in brain and have been shown to affect the brain neurotransmitter systems. Several of these hormones have also been shown to be responsible for specific behavioral effects.

Several neuroendocrine strategies are currently in use: (1) direct measurement in CSF and in plasma of behaviorally active peptides during the basal state and/or following stimulation according to verified stimulation paradigms; (2) administration of various hypothalamic releasing factors to test responses of the HP axis, and to elucidate patterns of monoaminergic disturbance and neuroendocrine dysfunction. For purposes of comparison and possible differential diagnosis, normal subjects, patients with affective illness, schizophrenia, and anorexia nervosa are studied: Some of the studies of peptide function are done with patients with Alzheimer's disease such as (3) psychoactive drug effects on HP function and on the levels of behaviorally active peptides; (4) assessment of the temporal organization of neuroendocrine function; and (5) assessment of the relationship between neuroendocrine function and sleep. We are also involved in studying the neurobiology of several neuroendocrine diseases, including Cushing's syndrome and Kallman's syndrome, and in developing clinical means for the differential diagnosis of Cushing's syndrome from depression and for distinquishing hypothalamic from pituitary Cushing's disease.

Because of limited laboratory support personnel and the large volume of assays reuqired to complete this neuroendocrine project, the Unit on Neuroendocrinology maintains collaborative and contractual arrangement with several university research facilities for neuroendocrine assays. We are fortunate to collaborate with scientists of outstanding caliber, including Dr. Seymour Reichlin, Dr. Samuel Yen, Dr. Gregory Brown, Dr. Gary Robertson, Dr. Detlev Ganten, and Dr. Delbert Fisher.

1. Studies of behaviorally active central nervous system (CNS) peptides:

a. We are continuing our comprehensive investigation of arginine vasopressin (AVP) function, concentrating on studies of CSF AVP, the plasma AVP response to hypertonic saline, and the cognitive and behavioral responses to vasopressin analog administration. We have also initiated a series of studies to examine non-osmolar stimuli to plasma secretion of AVP to directly evaluate

neurohypophyseal function. We have previously noted parallel changes in CSF AVP and the plasma AVP response to hypertonic saline in drug free subgroups of patients with affective disorder. Because CSF AVP is altered in the same direction as the sensitivity of osmoregulation, we have extended our studies of the neuromodulation of the osmoreceptor. We note that lithium increases the sensitivity but not the set of osmoregulation; thus, in the majority of subjects, lithium actually increases the sensitivity of the AVP response to hypertonic saline above a normal range. There have been no previously described clinical situations of pathological or pharmacologically-induced increases in the sensitivity of osmoregulation in human subjects. The physiologic effects of this increased sensitivity are proving to be subtle; the consequences of this altered sensitivity on CSF AVP and behavior are currently unknown. Carbamazepine diminishes the sensitivity of the AVP response to hypertonic saline and many patients actually showed a subnormal sensitivity to osmotic stimulation while receiving the drug. This subnormal sensitivity is compensated by a subtle shift to the right of the set of osmoregulation. Our study also documents that carbamazepine increases renal sensitivity to AVP. In susceptible patients, the subtle shift in the set of the osmoreceptor in association with sensitization to AVP effects would appear to contribute to the water intoxication occasionally noted in patients receiving carbamazepine. Zimelidine, a serotonin uptake inhibitor, increases the sensitivity of the osmotic response to AVP and also increases the level of CSF AVP. This finding represents the first pharmacological evidence that similar stimuli influence AVP secretion into CSF and plasma despite considerable evidence that AVP in CSF and plasma are derived from separate populations of neurons. Desmethylimipramine and naloxone do not influence the osmoregulation of AVP.

In collaboration with Dr. Walter Kaye and Dr. Michael Ebert, we have extended previous work on AVP function in patients with anorexia nervosa studied while chronically underweight and at intervals following correction of the weight loss. We now note that approximately 20% of chronically underweight patients show subnormal responses to hypertonic saline infusion, consistent with previous studies based on indirect methods suggesting partial nephrogenic diabetes insipidus in 40% of these patients. We also note that the remaining patients, who were thought to show normal regulation of AVP show a previously undescribed defect characterized by erratic secretion of AVP and a total dissociation of AVP secretion from osmotic stimuli. Most patients showed this newly described defect not only during the chronically underweight period but also several weeks after correction of weight gain. Abnormal osmoregulation is generally corrected by weight gain, but only if it is sustained for six months or more; some patients, however, show persistent osmoregulatory defects after normal weight is sustained for a year or more. Abnormalities of the osmoregulation of AVP were consistently associated with abnormalities in CSF AVP, particularly marked hypersecretion resulting in a reversal of the usual CSF/plasma gradient for AVP. This finding extends the evidence that CSF and plasma AVP are derived from separate populations of neurons but are regulated by analogous physiologic variables. Thus, the association of defects in the two compartments appears to be due to similar abnormalities in neurosecretory neurons or in controlling afferents.

In 12 drug free psychotic schizophrenic patients, CSF AVP was significantly lower than in 33 age matched normal volunteer subjects. This preliminary finding is currently being replicated. Osmoregulation has also been studied in 12 drug free schizophrenics; the data are currently analyzed.

Because of the cognitive properties attributed to AVP, we also studied the effects of DDAVP administration on cognition in patients with Alzheimer's disease. Such patients show failure to access and use previously learned knowledge (semantic memory) and have difficulty in encoding and processing ongoing events. DDAVP produced congitive enhancements in these patients through arousal related to facilitation of these semantic memory structures analogous to effects noted previously in depressed patients and control subject.

b. CSF levels of oxytocin were studied in collaboration with Dr. Delbert Fisher. Like AVP, oxytocin, (OT) is synthesized exclusively in hypothalamic nuclei and distributed widely in brain as well as transported to the posterior pituitary, where it is stored and subsequently released into the peripheral circulation. Fisher and his colleagues have demonstrated that a circadian rhythm exists for OT in CSF distinct from that of OT in plasma, suggesting a specific regulatory apparatus for secretion of OT into into CSF. OT is structurally very similar to AVP differing only in two amino acids. Recent data in experimental animals have suggested that OT may play a role in the CNS that is antithetical to that of AVP possibly secondary to direct competition of the two peptides for similar receptor sites. In experimental animals, some studies have shown that OT disrupts memory consolidation and retrieval; the findings are based on extinction rate and passive conditioned avoidance of responses. In addition, OT and AVP have been shown to exert opposite effects on hippocampal theta rhythms, reaction time to acoustical stimuli, shadow box avoidance, and spontaneous nerve activity. To date we have studied CSF OT levels in drug free patients with affective illness, schizophrenia, and anorexia nervosa. At present, our data show a state independent decrease in CSF OT in drug free bipolar patients during each of three clinical states: euthymia, mania, and depression. These preliminary data are currently in the process of replication. We cannot explain the observed decrease in CSF AVP and OT in bipolar depressed patients despite the presumed opposite CNS effects of AVP and OT. The relevance of the possible alteration in central OT function in patients with affective illness awaits further clarification.

c. CSF somatostatin (SS) levels were studied in collaboration with Dr. Robert Post, Dr. David Rubinow, and Dr. Seymour Reichlin. In a large number of patients there were no significant differences in CSF SS between drug free unipolar and bipolar depressed patients and controls. We have noted that a number of drugs influenced the secretion of SS into the CSF; zimelidine, the serotonin uptake inhibitor significantly increases SS levels in the CSF, while carbamazepine the antimanic and antidepressant agent significantly decreases SS secretion into CSF. We have also demonstrated a significant gradient for SS in CSF and shown that acidification of spinal fluid significantly increases recovery of SS immunoreactivity in CSF.

d. CSF levels of angiotensin I (AI), angiotensin II (AII), and angiotensinogen continue to be studied in collaboration with Dr. Detlev Ganten. Angiotensin is a central peptide that modulates fluid and electrolyte homeostasis. It influences learning processes in experimental animals and is intimately linked to central endorphin systems. In a preliminary study we have noted increases in the level of angiotensin I in patients with bipolar affective illness. This preliminary finding is apparently state independent. Schizophrenic subjects show normal mean levels of AI and AII. However, values in these subjects show a bimodal distribution a find deserving further investigation.

e. Neuroendocrine effects of opiate agonists and antagonists: We have previously reported that intravenous methadone administration diminishes plasma cortisol secretion in depressed patients. We now note that methadone also releases prolactin and vasopressin in depressed patients, but has no effect on growth hormone and gonadotropin secretion acutely. Naloxone has no effect on the AVP response to hypertonic saline or the TSH or prolactin response to TRH. We are currently studying neuroendocrine effects of opiate administration in both Cushing's disease and depression. We hypothesize that Cushings patients in contrast to depressed patients, may not suppress plasma cortisol levels following intravenous methadone, thus permitting differential diagnosis of these two entities by neuroendocrine means. This differential diagnosis is often difficult to establish at the stage of Cushing's disease before development of unequivocal physical stigmata and often results in considerable delays in treatment. Additional studies of depression and Cushings disease will be reviewed in a later section.

2. Dynamic tests of monoaminergically regulated HP systems:

We continue to evaluate the neuroendocrine responses to a three hour dopamine infusion in psychiatric patients. In collaboration with Dr. Gerry DeFraites, we have previously noted exaggerated gonadotropin responses, but normal prolactin responses to dopamine infusion in drug free schizophrenic patients, suggesting increased sensitivity of some hypothalamic dopamine receptors. Methodoligcally, this study also suggests that the gonadotropin rather than prolactin responses represent a usable neuroendocrine marker to assess the functional activity of median eminence dopamine receptors. Preliminary data suggest that the growth hormone response to dopamine infusion in schizophrenia is also exaggerated during the drug free period. There is also an increased growth hormone response to dopamine in patients studied 3 weeks after neuroleptic withdrawal which suggests that our findings in "drug free" schizophrenic patients may reflect increased sensitivity of dopamine receptors secondary to neuroleptic withdrawal.

Drug free patients with anorexia nervosa show blunted gonadotropin responses to sustained dopamine infusion. Metochlopromide infusion and naloxone infusion, both of which elevate gonadotropin secretion in patients with secondary hypothalamic amenorrhea do not appear to influence gonadotropin regulation in patients with anorexia nervosa, presumably because of refractoriness of unprimed gonadotrophs. We are currently pursuing studies that examine the interface between endogenous opiate systems and dopamine in anorexia nervosa.

3. Collaborative studies of the neurobiology of clinical neuroendocrine disease:

We have developed an extensive clinical and research collaboration with the Developmental Endocrinology Branch of the National Institute of Child Health and Development. Our neuroendocrine unit provides liaison coverage for the Developmental Endocrinology Branch Clinical Research Unit through Dr. Leslie Becker. He covers all clinical rounds and maintains regular formal contact with nursing and medical staff; in addition our unit conducts psychiatric evaluations, provides consultations for the unit and routine comprehensive psychiatric evaluations on patients with Cushing's syndrome, Addison's disease, Nelson syndrome, and Kallman syndrome in whom we have specific research interest.

We have a comprehensive neurobiological work-up of Cushing's disease, Addison's disease, Nelson syndrome, and Kallman syndrome. Extensive clinical evaluation, cognitive testing, neuroendocrine testing, circadian monitoring of activity temperature, EEG, AER and pain are included in this workup testing, and the evaluation of CSF peptides as well as biogenic amines and their metabolites. With respect to Cushing's disease we are currently analyzing these parameters in the hope of 1) establishing a reliable basis for the differential diagnosis of Cushing's disease from depression, and 2) establishing a reliable preoperative differential diagnosis between hypothalamic and pituitary Cushing's disease, and (3) comparing and contrasting pathophysiological features of Cushing's disease and depression thus furthering our understanding of each of the possibly related disorders. Thus, Dr. Gold will work directly with Dr. Lynn Loriaux in his laboratory to develop a radioimmunoassay for vasopressin and a bioassay using dispersed pituitary cells for corticotropin releasing factor.

Publications:

Gold, P.W. and Robertson, G.L.: Central peptide regulation of fluid and electrolyte balance: psychiatric implications. In Alexander, P. (Ed.): Electrolyte Disturbances in Neuropsychiatric Illness. New York, Spectrum Press, pp. 37-45, 1981.

Gold, P.W., Ballenger, J.C., Post, R.M., Weingartner, H.L., Robertson, G.L., and Goodwin, F.K.: Vasopressin in neuropsychiatric disorders. Special issue on psychoneuroendocrinology. In Brown, G.M. (Ed.): Int. J. Mental Health, 9:91-108, 1981.

Gold, P.W.: Contemporary endocrine-behavioral interrelationships. In Flach, F. (Ed.): Psychobiological Issues in Psychiatry, Monograph Series Vol. 4, Schering Corporation, New York, pp. 1-8, 1981.

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Gold, P.W., Post, R.M., Weingartner, H., and Goodwin, F.K.: Central peptide functions in affective illness. Arginine vasopressin as a model system. In Advances in Biological Psychiatry, deGroot (Ed.), Karger, Brussels, in press.

Gold, P.W., Ballenger, J.C., Weingartner, H., Post, R., Robertson, G.L., and Goodwin, F.K.: Vasopressin studies in affective illness: Levels in CSF, response to hypertonic saline and effects of chronic administration. In Neurobiology of Manic Depressive Illness, Post, R. and Ballenger, J.C. (Eds.), in press.

Pickar, D., Extein, I., Gold, P.W., Summers, R., Naber, D., and Goodwin, F.K.: Endorphines and affective illness. In Endorphines and Opiate Antagonists in Psychiatric Research: Clinical Implications, Shah, N.S. and Donald, A.G. (Eds.), Plenum Press, New York, in press.

Gold, P.W., Rubinow, D., Post, R.M., and Goodwin, F.K.: Neuroendocrine effects of psychotropic drugs. Annual Review of Toxicol. and Pharmacol., in press.

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Extein, I., Pickar, D., Gold, M.S., Gold, P.W., Potash, L., Sweeney, R., Ross, R., Rebar, R., and Goodwin, F.K.: Methadone and morphine in depression. Psychopharmcol. Bull. 17:29-33, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00449-07 CP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Outpatient Followup Studies of Manic-Depressive Patients and Families

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Yolande Davenport	Chief, Unit on Family Studies	CP/NIMH
	Rex Cowdry	Chief, Outpatient Unit	CP/NIMH
OTHER:	Frederick Goodwin	Chief	CP/NIMH
	William Thompson	Clinical Associate	CP/NIMH
	Philip Gold	Chief, Unit on Neuroendocrinology	CP/NIMH
	Marvin L. Adland	Consultant	CP/NIMH
	Sandra Bacon	Social Worker	CP/NIMH
	Thomas Wehr	Chief, Clinical Research Unit	CP/NIMH
	David Gardner	Psychiatric Resident	CP/NIMH
	Gay Grover	Clinical Nurse Expert	CC Nursing
	Leon Cytryn	Medical Officer (Research)	BP/NIMH
	Martine Lamour	Guest Worker	BP/NIMH
	Marion Yarrow	Chief	LDP/NIMH

COOPERATING UNITS (if any) Unit on Psychogenetics, BP/NIMH; Clinical Center Nursing; Extramural Clinical Research Branch, NIMH; McMaster University, Hamilton, Ontario; Parent Child, Inc., Wash., D.C.; UCLA, Los Angeles, California; LDP/NIMH; Univ. of Michigan, Ann Arbor, Mich.; Georgetown Univ., Washington, DC.

LAB/BRANCH
Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Long term outpatient lithium maintenance and psychotherapy groups involving bipolar patients or married couples with one bipolar member have provided a stable population for outpatient research efforts. Studies have emphasized (a) group dynamics and efficacy of group therapy with bipolar patients, (b) patterns of marital and family interactions associated with the illness, (c) child development and affective responses in infants with a bipolar parent. This year time-limited group psychotherapy has been a new focus of research, involving homogeneous populations of rapid-cycling patients with their spouses and homogeneous groups of unipolar depressed patients. A followup study of the outcome of rapid-cycling illness is nearly complete. Collaborative research into the phenomenology and psychobiology of seasonal affective disorders has begun.

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

Carolyne Waxler	Research Psychologist	LCP/NIMH
Elliot Gershon	Chief, Unit on Psychogenetics	BP/NIMH
Eleanor Dibble	Social Worker	BP/NIMH
Morris Parloff	Chief, Psychotherapy & Behavioral Interventional Center	CR/NIMH
Mariana Webster	Nurse Specialist	Parent Child
Gabrielle Carlson	Acting Director, Child Psychiatry Training Program	Neuropsych. Inst., UCLA
Catherine Connelly	Graduate Program Coordinator	Geo. Mason U.
John Nurnberger	Staff Fellow	BP/NIMH
Norman Rosenthal	Staff Psychiatrist	CP/NIMH

Project Description:

The outpatient program of the Clinical Psychobiology Branch is a collaborative project of the staff of the Unit on Family Studies and the Outpatient Unit. Over the last eight years, the outpatient program has focused primarily on providing continuing clinical care on an outpatient basis to patients previously studied on the inpatient units at NIH. Around this primary commitment to clinical care, a series of studies of therapeutic approaches to bipolar illness has coalesced. An extensive series of articles have been published describing the family and interpersonal dynamics which may develop around bipolar illness, and outlining our comprehensive medical and psychodynamic approach to treatment using group therapy. These "time-unlimited" psychotherapy groups consisting of bipolar patients or bipolar patients together with their spouses have proven to be an excellent approach to early identification and treatment of episodes and to long term work on dysfunctional patterns of interaction which may persist between episodes. As part of a thorough re-evaluation of the outpatient program, a decision was reached this year to discontinue these "time-unlimited" lithium maintenance and psychotherapy groups, since the major clinical and reseach goals of the project have been accomplished and since excellent resources are available in the community for continuing care of these patients. Several articles summarizing our long term work with these patients are in preparation.

A series of collaborative studies involving these patients will continue, including studies of patterns of family interaction and studies of the cognitive and emotional development of infants of bipolar patients. These are described in more detail below. Special subgroups of bipolar patients, such as individuals with rapid-cycling bipolar illness, will be a focus of increasing research. Outpatient followup studies are being expanded to include collaborative studies of the phenomenology and psychobiology of seasonal affective disorders. Regarding psychotherapy techniques, emphasis is now being placed on the advantages and limitations of time-limited group psychotherapy (four to six months) with homogeneous groups of patients, such as rapid-cycling patients and their spouses.

In addition to its traditional functions of clinical care and research, the outpatient program has instituted a training program in psychobiologic research for social work students and for psychiatric residents. During the coming academic year, four social work students will participate in a carefully supervised program of clinical assessment, psychotherapy, and research, with supervision from the Chief of the Unit on Family Studies. In addition, one psychiatric resident will spend half-time in the Outpatient Unit under supervision of the Chief. This training program is designed to illustrate the synthesis of the approaches of different disciplines, the ways in which clinical and biological research can synergize with rather than detract from clinical care, and an appreciation of the complex interactions between psychosocial factors and biologic predispositions.

Specific Projects

Although our project involving the treatment of married bipolar patients in conjunction with their spouses in group therapy is ending this year, the efficacy of this modality has been observed and described in previous papers. A summary of this work appeared this year as a chapter in a book on Major Psychopathology and Family Therapy. The therapeutic work with these patients appeared to interrupt the social isolation experienced by many bipolar patients and their families and to prevent rehospitalization through early recognition of stress and early treatment of incipient episodes. These long term groups provided a social continuity which seems important for these patients, as well as a setting in which faulty marital and family interactions could be explored and altered patterns of response could be suggested.

Two subgroups of bipolar illness have been particularly well studied through these couples groups. One weekly group has focused on the male bipolar patient with "dysphoric" manias. The data accumulated from the three year course of this group are still to be analyzed; however, it is apparent that patients who experience dysphoric manias may have distinctive character structure and defensive styles, persisting outside of the acute episodes, and expressing themselves both through recurrent themes in group psychotherapy and consistent patterns of interaction with spouses, parents, children, and superiors in work settings.

A study began this year of married rapid-cycling bipolar patients and their spouses, who were seen over a nine month period in a couples' psychotherapy group. The index patients, previously hospitalized on the inpatient unit, presented stormy histories of rapidly alternating manic and depressive episodes, each phase lasting two to six weeks. On admission to the group, the rapid-cycling patients were relatively stable on medication. However, marital problems, some of which were necessarily dormant during earlier periods of coping with the illness and rearing children, surfaced and were brought to the psychotherapy group for discussion. The purpose of the study was to observe whether specific differences existed in the defensive techniques of families with a rapid-cycling member in contrast to bipolar families previously studied where episodes occurred less frequently, less predictably, and with long periods of euthymia between episodes. The following major patterns and themes have emerged: families may develop a phenomenon we have termed "family cycling" in response to the cycling moods of the index patient; role diffusion is pronounced; fear of recurrence of the illness is more pervasive and anxiety producing in rapid-cycling families; and the degree of denial of affect dependency and loss is of the same magnitude found in other bipolar families. Although rapid-cycling illness is not rare, there is little in the literature describing the experience of these patients and their families, and the adjustments they make to the illness. The completed study will include a discussion of psychotherapeutic approaches to these families.

Female bipolar manic-depressive patients are considered at special risk for recurrence of a childbirth-related affective episode, as reported in a study of post-partum psychoses in a group of bipolar females followed in clinic. There are substantially fewer studies of the reactions of men to becoming fathers, although there is general acknowledgement of the fact that men undergo considerable emotional stress and that marital relationships are subject to

significant change at the time of a pregnancy. To test our hypothesis that episodes of manic-depressive disorder also occur in bipolar men in proximity to prospective fatherhood, a chart study was made of all former bipolar male patients admitted consecutively over a nine year period to our ward. Birth dates of all natural children and dates of hospitalization, as well as dates of outpatient episodes of mania and depression, were examined. The birth of a child was considered to have an influence on the occurrence of an episode when the episode occurred within nine months prior to or one year following the birth of the child. Family history for affective disorder, age of onset of affective illness in the patient, history of separation from the parent during the childhood years, and ordinal position in family of origin were among parameters examined. Of the 40 male probands studied, it was found that 21 patients had a total of 25 episodes during the pregnancy of the spouse or within one year post-partum. This is in marked contrast to the rare occurrence of childbirth-related psychoses among males in the general population. In contrast to reports of increased post-partum episodes found among women, the episodes found in the men tended to occur during the wife's pregnancy rather than following childbirth (16 pregnancy-related episodes compared to nine post-partum episodes). More of the episodes were manic than depressed; 17 episodes required hospitalization; eight episodes responded to outpatient treatment alone. The 21 men with birth-related episodes of illness tended to have a significantly earlier age of onset of manic-depressive disorder and a more severe illness than the 19 men without a pregnancy-related episode. These individuals also had a higher incidence of extended separation from parents during childhood. These data suggest that there may be a subgroup of male bipolar patients for whom fathering a child represents a sharply increased risk for perinatal or post-partum psychoses. Our findings strongly suggest the need for additional monitoring and ongoing psychotherapeutic support for both female and male bipolar patients during pregnancy and during the post-partum period. This additional attention may reduce stress, and it may enable early intervention to head off incipient affective episodes. Preventing affective episodes during this crucial period of a child's development may help ensure the physical and emotional availability of both mother and father to facilitate the processes of attachment, individuation, and separation in the growing child.

Data collection will be completed this summer in the collaborative study involving six infants born to parents followed in our lithium outpatient clinic. During the year, we were able to add an additional infant from our clinic. Thirty-six videotapes of 45 minutes duration which illustrate the development of affective responses in these infants at 12, 18, and 24 months have now been analyzed at the University of Colorado; the findings will be reviewed in a seminar of the study participants scheduled for this summer at the NIMH. The availability of videotapes of aged-match control infants will allow a more quantitative assessment of impressions that there are delays in motor and speech development and distinctive patterns of response to separation among some of the offspring of bipolar patients.

An ongoing interest in interactional patterns in families with an adolescent member with a psychiatric illness continued. A study measuring closeness preferences of adolescent offspring in schizophrenic, neurotic, and normal families is in press. This study suggests that the offspring in a family with a neurotic child describe attachments to the mother which are much closer than those described in normal families and among the well offspring in families with

a schizophrenic child. However, the schizophrenic patients surpass their own well siblings and comparable neurotic or normal offspring in their feelings of closeness to their mother. Within the families with a neurotic child, the intensities, splits, or coalitions observed affected all of the children in a somewhat similar pattern, even though one child in these families may be more neurotically disturbed than others. An extension of this study, using families having an adolescent with bipolar illness and at least one "normal" adolescent sibling, is planned in order to illuminate family closeness patterns within the "bipolar family" and to compare the pattern with that observed in the "schizophrenic," "neurotic," and "normal" families previously studied.

A study to examine history and outcome of 19 patients previously hospitalized on the inpatient unit with a rapid-cycling mood disorder is under way. The focus is on interim history since discharge from the inpatient unit, including the incidence of relapse and rehospitalization, the use of various treatment interventions, and the overall level of functioning achieved at the time of followup period. Preliminary data indicate that rapid-cyclers follow a highly variable course after discharge, but that at least three of these patients have independently discontinued all medications, with a marked improvement or cessation of their rapid-cycling affective disorder. Further results of our followup investigation will be reported in a summary article.

A study of shifting interdisciplinary roles on an inpatient psychobiological research unit was completed this year in collaboration with several members of the inpatient unit nursing staff. Five years ago, the psychotherapy program on the ward was restructured to give much greater emphasis to the role of primary nurse clinicians and to de-emphasize the role of individual therapy and management by the research physician. Since this change, only one patient has been discharged from the ward against medical advice, in contrast to 19 patients discharged against medical advice in the previous seven years. Paradoxically, this decrease in the number of discharges against medical advice occurred at a time when the number and strenuousness of research procedures in which a patient might participate during hospitalization had increased markedly. A retrospective study of characteristics of the 20 discharges against medical advice emphasized both the therapeutic role of the primary nurse and the increasing involvement of the patient as an active collaborator in the research as factors in minimizing discharges against medical advice. An article based on the study is in preparation.

Finally, the Unit on Family Studies is an active collaborator in three projects which have begun during the past year. The Unit will be providing psychosocial histories and clinical assessments of patients with seasonal mood cycles and patients who suffer from delayed sleep (phase lag) syndrome. The Unit will also be conducting time-limited group psychotherapy with borderline patients, to examine patterns of interpersonal and intra-familial interactions.

Publications:

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Targum, S.D., Dibble, E.D., Davenport, Y.B., and Gershon, E.S.: The family attitudes questionnaire: patients and spouses views of bipolar illness. Arch. Gen. Psychiatry 38:5 562-568.

Davenport, Y.B., and Adland, M.L.: Post-partum psychoses in female and male bipolar manic-depressive patients. Am. J. Orthopsychiatry. In press.

Hoover, C., and Davenport, Y.B.: Family closeness of young schizophrenics: patterns of closeness in the families of schizophrenic, neurotic, and normal adolescents. Am. J. Family Therapy. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01852-03 CP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Brief Outpatient Studies of Depressive Syndromes and Character Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Rex Cowdry	Chief, Outpatient Unit	CP/NIMH
OTHER:	Frederick Goodwin	Chief	CP/NIMH
	Yolande Davenport	Chief, Unit on Family Studies	CP/NIMH
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	Philip Gold	Chief, Unit on Neuroendocrinology	CP/NIMH
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	Arthur Behrmann	Resident in Psychiatry	Georgetown U.
	Gay Grover	Clinical Nurse Expert	CC Nursing
	Herbert Weingartner	Research Psychologist	LPP/NIMH
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COOPERATING UNITS (if any)
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SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	2	0.2

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Since the great majority of patients with depressive syndromes and character disorders are successfully treated in an outpatient setting, the Branch has placed a major emphasis on developing an outpatient facility for the clinical and biological evaluation and treatment of these patients. A comprehensive psychobiological evaluation of depression spectrum illnesses is performed, including psychological testing, circadian rhythm monitoring of temperature and activity, sleep electroencephalograms, TRH infusions, dexamethasone suppression tests, and collections of blood, urine, and cerebrospinal fluid. The treatment involves random assignment to either tranylcypromine or desipramine, with studies repeated following treatment with medication. A study of the neurophysiology of borderline syndromes, including a multi-drug cross-over study, has begun this year. In addition, small scale studies of the effects of tranylcypromine on rapid-cycling bipolar illness and the effects of a vasopressin analog on ECT-induced memory loss have been initiated. The role of lithium in the treatment of depressive pseudodementia and the occurrence of life-threatening pneumomediastinum as a complication of manic psychosis have been described in case reports.

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

David Goldstein	Asst. Prof. of Psychiatry	Georgetown U.

Project Description:

There has been increasing emphasis over the past several years in the study of the entire spectrum of depressive disorders, sparked by several findings. First, clinically homogeneous syndromes such as endogenous depression have been found to be biochemically heterogeneous, using tests such as the dexamethasone suppression test. Secondly, patients with non-endogenous depressions have been found to share some of the same biochemical abnormalities and drug responses as patients with more classical endogenous depressions. Third, specific behaviors, such as impulsive or suicidal behavior, which cut across diagnostic entities, have been associated with biological abnormalities such as low 5-hydroxyindoleacetic acid (5HIAA) in the spinal fluid. Because individuals with a wide spectrum of depressive disorders are commonly treated in outpatient settings, the outpatient program has developed a flexible and broad spectrum program for the evaluation of treatment of these disorders, requiring only brief inpatient stays. This approach helps assure a more representative sample of depressive disorders in our patient population, and reserves our inpatient resources for the longitudinal study of rapid-cycling and treatment-resistant patients.

The current evaluation and treatment program involves a screening, to assure the existence of a depressive syndrome that meets criteria for major depressive disorder. Hamilton scores of greater than 18 are required for admission to the study. If the patient agrees to participate, a more thorough clinical evaluation is carried out, including diagnoses of clinical states, description of character traits, and a series of assessments of relevant dimensions of depressive illness, including endogenicity, reactivity, and impulsivity. Psychological testing including an MMPI is performed. Biological parameters studied include circadian monitoring of temperature and activity, using recently developed solid state monitors. Sleep electroencephalograms are recorded on the inpatient unit to document sleep architecture. A lumbar puncture is performed, as are two neuroendocrine tests, the thyrotropin releasing hormone (TRH) test and the dexamethasone test (using 0.5 mg). Urine and plasma are collected for studies of neurotransmitters and their metabolites and neurohormones, and platelets are collected for platelet receptor studies. Following the week-long biological evaluation, the patient is entered in a double-blind random assignment manner to treatment with tranylcypromine or desipramine. Six to 10 weeks into treatment, at the time of maximal clinical response, the biological studies are repeated.

The decision to cast a very broad diagnostic net for the biological studies allows for the study of clinical and biological dimensions crossing traditional diagnostic lines. Thus it is possible to examine whether impulsivity and suicidality are traits related to specific biological findings, across the depressive spectrum of illnesses. In addition, this approach allows for careful study of the interrelationship of biological markers. For example, simultaneous circadian temperature monitoring and recording of sleep EEG allow for conclusions about the possible association of phase-advance in the temperature rhythm with decreased REM latency, both findings having been reported as markers for endogenous depression. Similarly, possible association between dexamethasone non-suppression and a blunted response to TRH infusion can be examined in the population as a whole. This comprehensive approach also allows for replication of previous small scale studies associating cerebrospinal fluid metabolite findings with specific neuroendocrine findings, such as a blunted response to TRH. Such associations may give additional insight into possible hypothalamic

regulatory mechanisms.

The treatment phase involving tranylcypromine and desipramine allows both for an evaluation of possible clinical or biological predictors of response and for the study of biological changes associated with treatment regardless of response.

To date, nine patients have completed both study phases of the program. Data analysis is ongoing and incomplete, but to date there have been no clear associations between biological findings and specific patterns of symptomatology. There does, however, appear to be a correlation between an advance in the circadian temperature rhythm and short REM sleep latency. In addition, preliminary data analysis suggests some association between failure of cortisol to suppress to 0.5 mg of dexamethasone and the occurrence of a blunted thyroid stimulating hormone (TSH) response to TRH.

A preliminary outpatient study of rapid-cycling bipolar affective disorder was begun, in part because of the limited availability of inpatient resources to treat these individuals. In view of our former findings of a very significant association between thyroid dysfunction and rapid-cycling illness, all patients entering into the outpatient study received a thorough thyroid evaluation, usually including a TRH infusion. Twice daily ratings were performed on visual analog scales by the patient, and ratings of overall depression or mania were made by the patients' spouses and by therapists. Preliminary results suggest that treatment with tranylcypromine, a monoamine oxidase inhibitor, can significantly alter the course of rapid-cycling illness, although the followup periods are as yet too short to state definitively whether the rapid-cycling has been terminated through medication treatment. More common than complete resolution of the cycling is a pattern in which either the severity of the mood cycle or the cycle duration is altered by the monoamine oxidase inhibitor. Further outpatient studies will be continued, probably involving an expansion of the inpatient studies using clorgyline, an investigational MAO type A inhibitor.

The Outpatient Unit and the Unit on Neuroendocrinology have been collaborating with the Fairfax Hospital in study of the effects of a vasopressin analog, DDAVP, on memory loss induced by electroconvulsive therapy. This study involves a comparison of DDAVP and placebo single administrations in reversing the ECT-induced memory loss, and uses a cross-over design with extensive tests of both free and cued recall. Results in the two patients studied to date are inconclusive.

The screening of possible participants in a long-term study of the borderline syndrome was begun this year. This study is based on a prior finding that individuals diagnosed as borderline have symptomatology suggestive of limbic system dysfunction and have a high incidence of abnormal, albeit largely non-specific, electroencephalograms. This study will examine electroencephalograms both in a routine clinical setting and under the stress of a semi-structured interview. In addition, the possibility of monoaminergic, particularly serotonergic dysfunction in these individuals with behavioral and affective dyscontrol will be examined through cerebrospinal fluid studies. A year-long double-blind placebo-controlled comparison of four medications suggested for use in the borderline syndrome (carbamazepine, alprazolam,

tranylcypromine, and trifluoperazine) will follow the completion of the evaluation study.

Two case studies based on experience in the Outpatient Unit have been written this year. The first reports a case of bipolar depressive pseudodementia, illustrating errors in interpreting "diagnostic" tests which led to a mistake in diagnosis of senile dementia, and reporting on the efficacy of lithium as an agent to treat both the depression and the pseudodementia in the elderly. The second case report documents the occurrence of a life-threatening pneumomediastinum as a result of Valsalva maneuvers performed by a young patient while in the acutely psychotic stage of a manic episode.

Publications:

Cowdry, R.W. and Goodwin, F.K.: The dementia of bipolar illness: Diagnosis and response to lithium. Am. J. Psychiatry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01831-05 CP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Basic and Clinical Studies of Neuronal and Glial Enolase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul J. Marangos	Chief, Unit on Neurochemistry	CP/NIMH
OTHER:	Frederick K. Goodwin	Chief, Clinical Psychobiology Br.	CP/NIMH
	J.M. Polak	Lecturer	Royal Med. Sch. London
	A.G.E. Pearse	Professor	Royal Med. Sch. London
	S. Bloom	Lecturer	Royal Med. Sch. London
	D. Schmechel	Neurologist	Duke Univ.
	John Minna	Oncologist	VAMO/ NCI
	D. Carney	Oncologist	VAMO/ NCI
	A. Gazdar	Biochemist	VAMO/ NCI
	M. Brightman	Head, Sec. on Neurocytology	LNNS/NINCDS

COOPERATING UNITS (if any)
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LAB/BRANCH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	0.6	1.1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies performed in our laboratory have established that a neuron specific and glial specific form of the glycolytic enzyme enolase exists in brain which have been designated neuron specific enolase (NSE) and non-neuronal enolase or NNE. During the past year studies have focused on the developmental profile of NSE and on the further study of the presence of NSE in paraneuronal APUD cells. Neurons switch from NNE to NSE during development a process which has now been shown to be correlated to synaptogenesis. NSE is therefore not only a unique marker for neurons it also represents a useful probe for studying neuronal differentiation. The APUD cells of the diffuse neuroendocrine system have also been further studied with virtually all lung, gut, pituitary, and skin neuroendocrine cells staining for NSE. Neuroendocrine tumors (APUDomas) also contain high levels of NSE in contrast to non-endocrine tumors which do not contain NSE. Clinical studies reveal highly elevated levels of NSE in oat cell carcinoma patients which decrease following chemotherapy indicating that NSE may be of diagnostic value.

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

G. Guroff	Chief, Sec. on Developmental Neurobiology	LDB/NICHHD
M. Whitehead	Res. Assoc./Pharmacology Dept.	Univ. of CT
G. Maxwell	Asst. Prof./Pharmacology Dept.	Univ. of CT
R.E. Fellows	CHmn. Phys. and Biophys. Dept.	Univ. of IA

Project Description

Proteins are the functional molecules of the cell since they serve to catalyze all biological reactions, function as receptors that recognize specific trophic agents such as hormones and neurotransmitters, regulate the accessibility of DNA, and specifically recognize other proteins to form specific structural arrays that represent the component parts of cells and tissues. The recent rapid advances in techniques for resolving and purifying proteins have revealed that not only do different cell types contain unique proteins but also that there are different forms of the same protein or enzyme (isoenzymes) in different types of cells. Since by definition, differentiated cells perform unique functions it is reasonable to assume that specific proteins have evolved to subserve these functions.

A major interest of our laboratory has centered on the isolation and characterization of cell specific proteins in brain. The motivating assumption is that the characterization of such proteins will provide very basic information concerning the distinctiveness of neuronal and glial physiology. We have in our laboratory determined that a neuronal and glial isoenzyme of the glycolytic enzyme enolase (E.C. 4.2.1.11) exists in brain. These proteins have been purified and extensively characterized both biochemically, immunologically and functionally, work summarized in previous years reports.

Extensive immunocytochemical analysis has established that the two major forms of enolase in brain are strictly segregated. The neuronal form is neuron specific enolase or NSE and the glial form is non-neuronal enolase or NNE. NNE and NSE are now quite well established as being the first useful cell specific markers for glial and neuronal cells respectively. NSE is present in all types of neurons and NNE in all types of glial cells.

The major focus of our work during the last year has been developmental studies, characterization of NSE in the diffuse neuroencodrine nervous system and clinical studies of NSE in various biological fluids of neurologic patients and endocrine tumor patients.

As described in previous annual reports we have shown that NSE is only present in differentiated neurons and that a genetic "switch" (takes place from NNE-NSE during neuronal development. This has turned out to be a very useful probe for the study of neuronal maturation. Using both our staining methodology and the specific radioimmunoassay we have developed for both NNE and NSE it is possible to study the development of specific areas in brain as well as of cells grown in culture. We have expended considerable effort during the past year in following up on the developmental studies mentioned in last year's report. The specific questions we are interested in concern the precise timing of the switch and what neuronal developmental stage it correlates with. In collaboration with Dr. Mark Whitehead and Kent Morest (University of Connecticut) we have studied the "switch" in the well characterized chick auditory system. The timing of all the neuronal developmental milestones are well characterized in this system and we utilized both staining and radioimmunological analysis of discrete brain areas in the developing chick embryo brain. This study is now completed and the results clearly show that in this system the onset of NSE immunostaining correlates quite closely with functional synapse development. Painstaking

dissection of these areas just before and just after synapse development and their analysis by radioimmunoassay supported the staining results quite nicely since NSE levels increase three fold during this time period. It therefore appears that the switch to NSE is correlated to synapse formation indicating that neuronal functioning i.e. depolarization, may be the triggering process for the switch. NSE therefore appears quite late in the developmental scheme of the neuron. Similar results were also obtained in developing ventral horn and dorsal root ganglion cells of the chick embryo. These studies were done in collaboration with Dr. Gerald Maxwell (University of Connecticut) and fully supports the results obtained in the auditory system, in that NSE could only be demonstrated in functioning neurons with established synaptic connections. Both of these studies have been submitted for publication to Developmental Brain Research.

We have devoted a major effort to the further study of NSE in neuroendocrine cells of the APUD series. As mentioned in last year's report we have shown that NSE is also present in peripheral neuroendocrine cells such as pinealocytes, pituitary corticotrophs, pancreatic islet cells and adrenal medullary chromaffin cells. These cells have been postulated to be neuronal relatives and have been designated either paraneurons or APUD (amine-precursor-uptake and decarboxylation) cells. The presence of NSE in these cells has supported the whole concept of these cells being neuronal relatives. In what has developed into an extensive collaboration with Dr. Julia Polak and A.G.E. Pearse (Royal Postgraduate Medical School, London) we have further explored the diffuse neuroendocrine nervous system in the gut, skin and lung. Both, staining and radioimmunoassay data have now firmly established that virtually all of the nearly 40 different peptides containing APUD cells contain NSE. For example, cells in the gut which contain either gastrin, somatostatin, VIP, or CCK all stain for NSE. Similarly, the neuroendocrine cells in the lung all contain NSE. The serotonin and bombesin containing cells in lung also stain for NSE and perhaps the most interesting finding of this study is that there are a population of cells in lung that stain only for NSE and not for bombesin or serotonin. The same is found in the gut and pancreas which indicates that there may be quite a few peptide secreting APUD cells which remain to be characterized. The lung work has been completed and published in the American Journal of Pathology and the work describing NSE in the gut has been submitted to Gastroenterology. Extensive immunocytochemical analysis of various tissues is in progress. A study has been completed describing the presence of NSE in Merkel cells of mammalian skin. These neuroendocrine APUD cells have been extremely difficult to study due to their sparse distribution. Their intense stainability with anti-NSE serum therefore makes feasible the study of these cells. This study has been accepted for publication in the American Journal of Pathology.

The peptides produced in the APUD cells that constitute the diffuse neuroendocrine nervous system are also found in brain and it is thought that the peripheral APUD cells communicate with the brain by an endocrine link with the peptide carrying the message. This system of communication probably serves to maintain homeostasis in the respective organ systems with the brain performing the coordinating functions. NSE is a general marker for all these cell types and will therefore be of tremendous value in characterizing this newly discovered division of the nervous system.

From the clinical standpoint during the past year we have shown that all the endocrine tumors of APUD cells (APUDomas) also contain high levels of NSE. In this regard, we have analyzed biopsy tissue from pheochromocytomas, glucagonomas, insulinomas, oat cell carcinomas, etc. All these APUDoma tissues show high NSE levels by both radioimmunoassay and immunocytochemical staining. This represents a significant advance in the ability to type tumors as APUDomas since although some produce their respective peptide product this is very variable. We have analyzed 84 APUDomas and 12 non-endocrine tumors, all of the former contained high levels of NSE while none of the latter had significant NSE. This work has been published in Lancet.

In an effort to determine whether serum from APUDoma patients contained elevated NSE levels we have adapted our ^{125}I radioimmunoassay for serum and plasma determinations. We have thus far examined sera from pheochromocytoma, medullary thyroid carcinoma, and oat cell carcinoma. Only in the oat cell patients were elevated NSE levels observed. These studies done in collaboration with Drs. John Minna, Adi Gazdar and Desmond Carney of the National Cancer Institute have shown that serum NSE levels are correlated to the degree of tumor bulk and that of the three types of lung cancer (squamous cell, adenocarcinoma and oat cell carcinoma) only oat cell patients have elevated NSE levels. In a group of 16 patients NSE levels were determined during the clinical course of the illness. In these studies virtually all patients showed a marked decrease in NSE following chemotherapy and dramatic elevations during remission. Normal serum levels of NSE range from 4-12 ng/ml of serum or plasma while levels in oat cell patients range from 40 to 850 ng/ml. We have also determined NSE levels in oat cell cultures obtained from patients and shown extremely high levels of the protein. It therefore appears that NSE determinations in serum can be very useful in the diagnosis of oat cell carcinoma as well as in the evaluation of response to treatment. These studies are being submitted for publication to Lancet. We are also currently screening a variety of other APUDoma clinical material and have obtained preliminary results suggesting that NSE may be elevated in the serum of glucagonoma patients.

Other clinical studies that have been done during the last year include an analysis of NSE and NNE levels in various brain areas obtained at autopsy from Huntington's disease patients. In these studies a 50% decrease in NSE is observed in the caudate of Huntington's patients while cerebral cortical levels remained unchanged. NNE levels were not substantially altered in either brain area. These results indicate that NSE can also serve as a valuable index of neuronal degeneration for various neuropathological studies. This study is in press in the Journal of Neurochemistry.

The neuronal and glial enolases represent a unique system in neurobiology in that they are the first example of cell specific multiple forms of the same enzyme in brain. The immunocytochemical and radioimmunological methods we have developed using the antisera to NSE and NNE confer the capability to perform a wide range of studies where data concerning the amount and identity of neurons and glia are required. The switch from NNE to NSE during differentiation also provides a unique probe for the study of neuronal differentiation and aging. The universal presence of NSE in apparently all paraneuronal APUD cells provides

the first simple means of characterizing the diffuse neuroendocrine nervous system. Immunohistochemical studies of NSE distribution should also serve to identify new peptide secreting cells and provide important insights concerning the actual extent of the diffuse neuroendocrine nervous system.

Publications:

Facer, P., Polak, J.M., Marangos, P.J. and Pearse, A.G.E.: Immunocytochemical localization of neuron specific enolase (NSE) in the gastrointestinal tract. Proc. Roy. Microscop. Soc. 132:113-114, 1980.

Cole, G.A., Polak, J.M., Wharton, J., Marangos, P.J., and Pearse, A.G.E.: Neuron specific enolase as a useful histochemical marker for the neuroendocrine system of the lung. J. Pathol. 132:351-352, 1980.

Marangos, P.J., Schmechel, D.E., and Oertel, W.: Neuron specific enolase, a specific functional marker for the diffuse neuroendocrine system. In Gut Hormones. Second Edition, Bloom, S.R., and Polak, J.M. (Eds.). Churchill-Livingstone, Edinburgh, p. 101-106, 1981.

Tapia, F.J., Polak, J.M., Barbosa, A.J.A., Marangos, P.J., Bloom, S.R., Dermody, C., and Pearse, A.G.E.: Neuron specific enolase is produced by neuroendocrine tumors. Lancet, i:808-812, 1981.

Parma, A.M., Marangos, P.J., and Goodwin, F.K.: A more sensitive radioimmunoassay for neuron specific enolase (NSE) suitable for cerebrospinal fluid determinations. J. Neurochem. 36:1093-1096, 1981.

Trapp, B.D., Marangos, P.J., and Webster, H. DeF.: Immunocytochemical localization and developmental profile of neuron specific enolase (NSE) and non-neuronal enolase (NNE) in aggregating cell cultures of fetal rat brain. Brain Res., in press.

Jiang, G.U., Polak, J.M., Tapia, F.J., Marangos, P.J., and Pearse, A.G.E.: Neuron specific enolase in the Merkel cells of mammalian skin. American J. of Pathology, in press.

Wharton, J., Polak, J.M., Cole, G.A., Marangos, P.J., and Pearse, A.G.E.: Neuron specific enolase as an immunocytochemical marker for the diffuse neuroendocrine system in human fetal lung. J. of Histochem. and Cytochem., in press.

Vinores, S.A., Marangos, P.J., Parma, A.M., and Guroff, G.: Increased levels of neuron specific enolase in PC 12 Pheochromocytoma cells as a result of nerve growth factor treatment. J. Neurochem., in press.

Askanas, V., Marangos, P.J., and King Engel, W.: CSF from amyotrophic lateral sclerosis patients applied to motor neurons in culture fails to alter neuron specific enolase. Neurology, in press.

Marangos, P.J.: Basic and clinical neurobiological applications of neuronal (NSE) and non-neuronal (NNE) enolase. Advances in Clinical Enzymology, in press.

Publications:

Campbell, I.C., Marangos, P.J., Murphy, D.L., and Pearse, A.G.E.: Neuron specific enolase (NSE) in human blood platelets: Implications for the neuronal model. CINP, in press.

Marangos, P.J., and Paul. S.M.: Brain levels of neuron specific and non-neuronal enolase in Huntington's disease. J. Neurochem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01833-01 CP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Adenosine Receptors in the Central Nervous Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	M. Brightman	Head, Sec. on Neurocytology	LNNS/NINCDS
	Jacqueline N. Crawley	PRAT Fellow	CP/NIMH

COOPERATING UNITS (if any)
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	0.4

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Adenosine is a potent neuromodulator in brain having marked inhibitory effects on neuronal firing and the activity of adenyl cyclase. The recent availability of two metabolically stable adenosine analogues N^6 cyclohexyl-[^{3}H] adenosine, [^{3}H] CHA, and [^{3}H] diphenyl xanthine, [^{3}H] DPX has made possible the direct analysis of adenosine receptors in brain. We have characterized the binding of these compounds in brain showing two binding sites for [^{3}H] CHA with affinity constants of 0.7 and 2.4 nM and one binding site for [^{3}H] DPX (K_d = 6.2 nM). The metals copper and zinc have been shown to be highly potent inhibitors of [^{3}H] CHA binding while both calcium and magnesium increase binding. Developmental studies show that [^{3}H] CHA and [^{3}H] DPX binding sites appear between 5 and 10 days after birth with cerebellar receptors developing sooner than those in the forebrain. A method has also been devised for the autoradiographic visualization of adenosine receptors and their distribution has been mapped. Especially high levels of receptor are seen in the superficial layer of the superior colliculus and in the cerebellar cortex.

Project Description:

Numerous studies have indicated that adenosine play a key role in the central nervous system as a neurotransmitter or neuromodulator. This ubiquitous purine has marked depressant effects on neuronal firing as well as on the activity of adenylate cyclase. Adenosine can either increase or decrease the level of cyclic AMP a phenomenon thought to be mediated by either A_2 or A_1 receptors respectively. Substantial evidence indicates that adenosine, which is released in the form of ATP during neurotransmitter exocytosis, acts by inhibiting presynaptic release of transmitters. The mechanism of such an action is currently not known. There is also a specific reuptake system for adenosine which seems to be distinct from the adenosine receptor since reuptake inhibitors are very poor inhibitors of the adenosine receptor.

Studies concerning the function of adenosine in brain have been severely hampered for several reasons. Brain tissue homogenates and membrane fractions generate adenosine at a fairly rapid rate making studies utilizing [^{3}H] adenosine difficult since the endogenous level is high. The high endogenous adenosine levels can be dealt with by incorporation of the enzyme adenosine deaminase which converts adenosine to inosine but such a treatment also destroys the labeled adenosine. This problem has recently been solved by the synthesis of labeled adenosine analogues which retain their ability to bind to adenosine receptors but are not substrates for adenosine deaminase. Two such compounds are N^6 cyclohexyl-[^{3}H] adenosine [^{3}H] CHA and [^{3}H] diphenylxanthine, [^{3}H] DPX. Synaptosomal membranes prepared with adenosine deaminase can therefore be analyzed for adenosine receptors using these two ligands.

We have initiated a broad spectrum of studies using these valuable new, metabolically stable adenosine receptor ligands with the purpose of further characterizing this system at the biochemical, pharmacologic and behavioral level. The biochemical and pharmacologic studies have been greatly facilitated by the coming of Dr. Jitendra Patel to our Branch as a Fogarty International Fellow. Dr. Patel did his doctoral work on adenosine receptors at the University of London with Dr. Henry McIlwain. The behavioral studies have been done in collaboration with Dr. Jacqueline Crawley who is a PRAT fellow.

We have during the last six months characterized the binding of both [^{3}H] CHA and [^{3}H] DPX to the adenosine receptor in rat brain. Saturable high affinity binding is seen with both ligands although the kinetics are quite distinct. [^{3}H] CHA binding shows two components with K_d values of 0.7 and 2.4 nM and respective B_{max} values of 230 and 120 fmoles/mg protein. The indication is that the two ligands have different binding capacities and may be interacting with different adenosine receptor subclasses. Copper and zinc have been shown to be potent inhibitors of [^{3}H] CHA binding with respective IC_{50} values of 30 and 150 μM. The physiologic significance of this finding remains unknown. Regional distribution studies indicate a high concentration of adenosine receptors in the hippocampus with lower levels in cerebral and cerebellar cortex and quite low levels in medulla and spinal cord. The receptor characterization has been completed and submitted for publication to Brain Research.

In an effort to determine the time of appearance of adenosine receptors we have done a developmental study where forebrain and cerebellar tissue from rats ranging in age from the 18th embryonic day to adult were analyzed for adenosine receptors. Results indicate that the ontogeny of the adenosine receptor parallels that of neuronal differentiation and the phylogeny of the various brain areas. Phylogenetically older areas of brain such as cerebellum display a more rapid acquisition of adenosine receptors than areas such as cerebral cortex. Similar results are seen with both [^{3}H] CHA and [^{3}H] DPX binding analysis.

We have also developed methods for labeling adenosine receptors on frozen sections and visualizing hem autoradiographically using tritium-sensitive film. Distinct localizations of [^{3}H] CHA binding are seen which make possible the precise mapping of adenosine receptors in brain. Computer color coding using relevant standards also makes possible direct quantitations of the receptor. The receptor is localized to various layers of the cerebellar cortex whereas it is more diffusely localized in the cerebral cortex. No receptors are seen in areas rich in glia such as the corpus callosum. A very marked localization of adenosine receptors is seen in the superficial layer of the superior colliculus. The preliminary localization study of adenosine receptors using our autoradiographic technique has been submitted for publication to the European Journal of Pharmacology.

The authoradiographic technique is also being used to trace the pattern of appearance of the receptor during development. These studies are in progress and are expected to complement the biochemical study on ontogeny of the receptor. The sequence of appearance of adenosine receptors during development should furnish insight concerning the functional pathways modulated by this purine.

A wide range of studies are planned using the autoradiographic visualization procedure. The results obtained thus far have established that contrary to the ubiquitous presence of adenosine in all brain areas, the receptors are only present in restricted areas. Experiments are planned where various pharmacologic and surgical manipulations will be assessed for their effects on the autoradiographic distribution of the receptor. This powerful anatomical tool is now being incorporated into all of our receptor related studies in the laboratory and is expected to provide valuable information relating to functional pathways.

Behavioral effects of adenosine have been difficult to characterize since an administered dose of this purine is rapidly metaolized by adenosine deaminase or various nucleoside processing enzymes. The metabolically stable adenosine analogues such as CHA and 2-chloradenosine, however, do not suffer from this drawback. We have therefore studied various simple behavioral endpoints such as activity, sedation, and ability to reverse diazepam induced increase in exploratory behavior. Both had sedating effects at doses of 0.1 to 0.5 mg/kg while the doses of adenosine required to produce similar effects were 2-3 orders of magnitude higher. A similar dose relationship was also found for the reversal of diazepam induced increase in exploratory activity with adenosine having a marked effect at 20 mg/kg and the analogues being active at 0.01 to 0.1

mg/kg. Both CHA and 2-chloroadenosine are both extremely potent as sedatives compared to the benzodiazepines, opiates, barbiturates and phenothiazines which are all 10-100 fold less potent. The preliminary behavioral analysis of the metabolically stable adenosine analogues has been submitted for publication to Life Sciences.

An additional area of interest concerning the adenosine system relates to the mechanism of action of the methylxanthine stimulants such as caffeine and theophyline. We have shown that these compounds competitively inhibit [^{3}H] diazepam binding. They also inhibit [^{3}H] CHA binding suggesting that these agents may interact with both the benzodiazepine and adenosine systems in brain. A rigorous analysis of the inhibitory potency of the methylxanthine stimulants on [^{3}H] CHA, [^{3}H] DPX and [^{3}H] diazepam binding is therefore in progress in an effort to determine which receptor system is preferentially effected by these agents. These studies are being done on human cortical membranes and all ligands are being tested on the same membrane preparation. Preliminary results suggest that the methylxanthines are more potent in their interaction with the adenosine receptor. Detailed analysis of the interaction of caffeine with adenosine and benzodiazepine receptors in the same membrane preparations should provide important information concerning the interrelationship of these two receptor systems. Work in our laboratory has already shown that various purines are able to inhibit [^{3}H] diazepam binding although the benzodiazepines do not inhibit [^{3}H] CHA binding. The relationship of these two systems is therefore likely to be quite complex. Studies have also been recently initiated using [^{3}H] nitrobenzylthioinosine. This compound has been reported to bind selectively to the adenosine reuptake site in Hela cells and red blood cells. We are characterizing its binding in brain membranes and will be investigating the relationship of this binding site to both the adenosine and benzodiazepine receptors.

Further characterization of the adenosine receptor and its functional role in the central nervous system may lead to the development of new pharmacologic agents and will certainly provide insight concerning the modulation of neuronal activity.

Publications:

Lewis, M.E., Patel, J., Moon-Edley, S., and Marangos, P.J.: Autoradiographic visualization of rat brain adenosine receptors using N^6 cyclohexy [^{3}H] adenosine. Europ. J. Pharmacol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01834-04 CP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Endogenous Ligands for the Brain Benzodiazepine Receptor

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1.4	0.8	0.6

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is now established that the benzodiazepines exert their actions via the specific receptor site in brain that binds these drugs with high affinity. Studies in our laboratory have focused on the characterization of the receptor and the elucidation of endogenous ligands. In this regard, the purines inosine and hypoxanthine have been further characterized and new studies involving melatonin and its brain metabolite N-acetyl-5-methoxy-kynurenamine have shown that these compounds are also putative endogenous ligands for the benzodiazepine receptor. Studies involving β-carboline-3-carboxylate ethyl ester (β-CCE) show that this ligand reacts differently with the receptor than antagonists (benzodiazepines), in that its binding is modulated by GABA and that it is more specific for the "central type" benzodiazepine receptor. We have also shown that β-CCE is more specific for the central type benzodiazepine receptor since very low binding is observed in kidney membranes.

Project Description

Biologically active agents such as hormones and neurotransmitters probably initiate their actions by binding to specific recognition sites or receptors. Recognition of ligands by specific receptors imparts specificity to biological interactions and can be viewed as the first link in a biological cascade of discrete molecular events culminating in a cellular response. Utilizing receptor binding strategies provides a simple means of characterizing various receptor-ligand systems in brain as well as providing information concerning the interactions of drugs with various neurotransmitter and neuromodulator systems.

The benzodiazepines probably exert their pharmacologic actions via the benzodiazepine receptor since the potency of these agents as anxiolytics and anticonvulsants correlates well with their receptor binding potency. This binding site therefore probably represents the initial and primary site of action of these drugs. The major inhibitory neurotransmitter GABA also has marked effects on benzodiazepine binding suggesting that the two systems are coupled. The existence of specific receptors for non-physiological agents such as the benzodiazepines predicts that endogenous ligands exist in vivo. The focus of our research on the benzodiazepine system has therefore concerned the interactions of the receptor with the GABA system and the elucidation and characterization of putative endogenous ligands for the benzodiazepine receptors.

Studies concerning the effects of chronic clonazepam and chlordiazepoxide treatment on the benzodiazepine and GABA receptors have been completed during the past year. These two benzodiazepines were chosen since they represent the opposite ends of the spectrum concerning affinity for the receptor. Clonazepam is the most potent benzodiazepine (IC_{50} = 1 nM) while chlordiazepoxide is one of the least potent (IC_{50} = 1000 nM). In these experiments the animals chronically treated with clonazepam developed receptor subsensitivity in that the number of available benzodiazepine receptors decreased. In a companion study it was shown, in the same animals, that the number of GABA receptors increased in a manner quite reciprocal to the decrease in benzodiazepine sites. These studies indicate that the two receptors (benzodiazepine and GABA) are coupled and support previous behavioral experiments showing a facilitation of GABAergic effects by benzodiazepines. Both of these projects are in press in Neuropharmacology.

Studies have also been performed during the last year concerning the mechanism by which GABA increases benzodiazepine binding. In these studies three parameters were examined simultaneously; (1) diazepam binding, (2) muscimol binding and (3) GABA stimulated diazepam binding. Conditions were devised where muscimol binding was selectively effected and the effect on GABA stimulated binding was examined. The overall interpretation of these studies was that the enhancement of diazepam binding by GABA is not mediated by the high affinity GABA receptor site. The best explanation of our results is that GABA interacts directly with the benzodiazepine receptor in an allosteric manner. This interpretation is supported by the fact that the solubilized partially purified benzodiazepine receptor is still modulated by GABA. These studies are in press in Molecular Pharmacology.

Our work on endogenous ligands has advanced during the past year. Due to the difficulties involved in processing large quantitites of tissue and purifying unknown substances our strategy has changed somewhat. We have begun examining known compounds with structures similar to those which have already been shown to inhibit diazepam binding. Both, tryptophan and the derivative β-carbolines have been shown to be active at the benzodiazepine receptor. Our efforts during the last year have therefore centered on the characterization of tryptophan derivatives with stress on the β-carbolines.

We have screened all naturally occurring tryptophan derivatives and found that melatonin and its brain metabolite N-acetyl-5-methoxy kynurenamine (AMK) are competitive inhibitors of diazepam binding. Melatonin has an IC_{50} of 500 μM while AMK is approximately 10 fold more potent with an IC_{50} of 50 μM. Both are competitive inhibitors and are not active on opiate, cholinergic, adrenergic or GABA receptors indicating that they are highly specific. This work is in press in Life Sciences and is currently being elaborated on by trying to determine the levels of AMK and melatonin in brain. This information will be critical before any physiological relevance can be associated with our findings. Melatonin is closely related in structure to the β-carbolines which have been shown to be potent inhibitors of diazepam binding. A simple cyclization of the side chain yields 6-methoxy-norharman which we have shown does inhibit diazepam binding with a potency similar to AMK. We are currently investigating whether this reaction occurs *in vivo*.

The compound β-carboline-3-carboxylate ethyl ester (β-CCE) has been shown in Braestrup *et al.* to be a very potent inhibitor of diazepam binding (IC_{50} = 7 nM). [^{3}H] β-CCE has recently become available and we have fully characterized the binding of this compound to the benzodiazepine receptor. These very interesting studies have shown that in marked contrast to [^{3}H] diazepam binding, that the bind of [^{3}H] β-CCE is not stimulated by GABA. This is therefore the first benzodiazepine receptor ligand for which GABA has no modulatory effect on binding. Since β-carbolines have opposite effects compared to the benzodiazepines (i.e. are convulsants and antagonize the sedation induced by flurazepam) they can be considered agonists and the benzodiazepines antagonists. It therefore appears that agonist binding is not modulated by GABA suggesting that agonists and antagonists interact in a unique manner. We have also kinetically characterized [^{3}H] β-CCE binding and shown it to bind to apparently one population of binding sites with a K_d of 1.4 nM. Our studies suggest that [^{3}H] β-CCE is acting on the same population of receptors as diazepam. An additional interesting observation is that [^{3}H] β-CCE is much more selective in its interaction with the "central type" benzodiazepine receptor since it binds very poorly to kidney membranes which contain "peripheral type" benzodiazepine receptors. This ligand should therefore prove useful in studies concerning the relationship of peripheral and central type receptors. The studies concerning [^{3}H] β-CCE binding are in press in the European Journal of Pharmacology with an additional manuscript detailing the kinetic parameters of binding having been submitted for publication to Life Sciences. It is doubtful whether β-carboline derivatives will represent clinically useful drugs since their activating, convulsant properties are not desirable. However, the value of these agents as probes for the study of the benzodiazepine receptor system is likely to be considerable.

Two years ago we reported that purines were active in inhibiting diazepam binding and have since shown that these compounds have pharmacologic, neurophysiologic and behavioral properties that support their postulated involvement with the benzodiazepine system. Studies concerning the characterization of various naturally occurring purines as putative endogenous ligands have continued this year. In collaboration with Dr. Crawley we have shown that inosine reverses the increase in activity seen in mice in response to low doses of diazepam. The doses of inosine used in this study were relatively low (20-50 mg/kg) in contrast to the higher doses which we have previously shown to be anticonvulsant. These studies show that inosine does specifically effect benzodiazepine mediated behaviors and has been published in Science.

We have also completed studies concerning the inhibition of caffeine-induced seizures by the benzodiazepines and inosine. These experiments have shown that inosine has diazepam-like effects in this system and further suggests that purines such as inosine may represent naturally occurring compounds that antagonize the cortical stimulant effects of the methylxanthines. These studies have recently been published in Psychopharmacology. As a follow-up study we have also measured inosine levels in microwave sacrificed mouse brain before and after inosine administration. An HPLC assay for purines was devised by Dr. Trams who collaborated in this effort. The results of this study showed that inosine levels were elevated 3-4 fold in brain following an anticonvulsant dose of inosine and more importantly that these levels were sufficient to significantly inhibit diazepam binding. It is therefore plausible to assert that the anticonvulsant effect of inosine is due to its effect on benzodiazepine receptors. These studies are in press in Psychopharmacology.

As is the case in most areas the rapidly expanding data base concerning the physiology of the benzodiazepine receptor has generated a complex conceptualization of the function of this system in brain. It is now postulated that several receptors may exist and the list of putative endogenous ligands is certainly becoming quite extensive. It is possible that separate systems exist which mediate the different pharmacologic effet of the benzodiazepines, i.e. anxiolytic, anticonvulsant, sedative hypnotic and muscle relaxant. It is also postulated that the benzodiazepine receptor, the GABA receptor and the chloride ionophore exist as a macromolecular unit although no direct evidence supports this. Whether the benzodiazepine receptor represents the site of action of a heretofore undescribed neurotransmitter or neuromodulator is also uncertain. Future claims concerning the physiological relevance of this system will depend on the demonstration and characterization of both biochemical and electrophysiologic responses to endogenous ligands for the receptor.

Publications:

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Crawley, J.N., Marangos, P.J., Stivers, J., and Goodwin, F.K.: Chronic clonazepam administration induces benzodiazepine receptor sensitivity. Neuropharmacology, in press.

Marangos, P.J., and Crawley, J.M.: Chronic benzodiazepine treatment increases [^{3}H] muscimol binding in mouse brain. Neuropharmacology, in press.

Marangos, P.J., and Martino, A.M.: Studies ont he relationship of GABA stimulated diazepam binding and the GABA receptor. Mol. Pharmacol., in press.

Marangos, P.J., Paul, S.M., Parma, A.M. and Skolnick, P.: Inhibition of GABA stimulated diazepam binding by benzodiazepine receptor ligands. Biochem. Pharmacol., in press.

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Marangos, P.J., Patel, J., Hirata, F., Sondhein, D., Paul, S.M., Skolnick, P., and Goodwin, F.K.: Inhibition of diazepam binding by tryptophan derivatives including melatonin and its brain metabolites N-acetyl-5-methoxy-kynurenamine. Life Sci., in press.

Patel, J., Marangos, P.J. and Goodwin, F.K.: [^{3}H] Ethyl-β-carboline-3-carboxylate binding to the benzodiazepine receptor is not effected by GABA. Europ. J. Pharm., in press.

Marangos, P.J., Trams, E., Clark-Rosenberg, R.L., Paul, S.M. and Skolnick, P.: Anticonvulsant doses of inosine result in brain levels sufficient to inhibit [^{3}H] diazepam binding. Psychopharmacology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01836-03 CP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Receptors for Neurotransmitters, Neuropeptides, and Neuromodulators in the Central Nervous System

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5.7	3.4	2.3

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Recognition sites (receptors) present in the central nervous system are physiologically modulated by endogenous transmitters and modulators. Furthermore, it is believed that many psychotropic drugs exert their therapeutic actions by affecting these receptors. Several receptor systems are currently under study including receptors for benzodiazepines, tricyclic antidepressants, adenosine and the neuropeptide cholecystokinin. The role and regulation of these systems are investigated by: 1) Characterization of these recognition sites. 2) Characterization of endogenous modulators of these receptor sites. 3) Development of appropriate in vitro models for these receptors. 4) Structure-activity studies of chemically modified substances which are related to the naturally-occurring ligands of these receptor sites. 5) Behavioral and pharmacologic experiments in animals combined with an examination of subsequent alterations in the character of these receptor sites. 6) Genetic studies in inbred strains of rats and mice coupled with examination of receptor changes as the correlate to gross behavioral manifestations of these genetic traits. 7) Clinical studies on receptors present in readily accessible peripheral tissues of man.

Names, Laboratory and Institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

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Project Description:

Objectives: To characterize and determine the role and regulation of receptors for tricyclic antidepressants, benzodiazepines, adenosine and cholecystokinin, in the central nervous system. To isolate, identify and characterize endogenous substances which physiologically modulate these receptor sites. To examine the effects of physiologic, pharmacologic, and behavioral manipulations on the plasticity of these recognition sites. To develop potential therapeutic agents and neurochemical tools using information obtained from the above studies.

Methods Employed: Radioreceptor assay the primary tool in neurochemical characterization of receptor sites and as a means of detecting platelet and brain membranes (rodent and human) were used in studying tricyclic antidepressant binding ([^{3}H] imipramine and [^{3}H] desipramine). Brain membranes and brain slices have been used to study the binding of [^{3}H] benzodiazepines ^{125}[I]-cholecystokinin and [^{3}H] cyclohexyladenosine (CHA), a stable adenosine derivative. Radioenzymatic techniques combined with thin-layer chromatography were used to examine the role of adenosine deaminase in the sedative/hypnotic actions of 9-(erthyrohydroxynonyl)adenine (EHNA), an inhibitor of adenosine deaminase. High pressure liquid chromatography, gel filtration, and membrane filtration have been used to characterize endogenous compounds. Pharmacologic testing of compounds has been accomplished through two procedures. Anticonvulsant/proconvulsant activity was assessed using sensitivity to the convulsant pentylenetetrazole (PTZ). A newly designed behavioral apparatus which measures the activity of mice in a novel environment was employed in examination of anxiolytic activity as well as for disruption of the anxiolytic action of compounds such as the benzodiazepines.

Major Findings: Saturable, high affinity binding of radiolabelled tricyclic antidepressants have been characterized in both rat and human brain as well as human platelets. Chemical and electrolytic lesions of the brain, the inhibition of [^{3}H] imipramine binding with 2,8-dinitroimipramine (an irreversible inhibitor), and competition studies with a series of pharmacologically active secondary and tertiary amine tricyclic antidepressants were used to evaluate the nature of this binding site. The results strongly suggest that [^{3}H] imipramine labels a site in rat brain, human brain, and human platelet which is an integral component of the transport site for serotonin. In contrast, high affinity [^{3}H] desipramine binding labels sites associated with the uptake of norepinephrine in rat brain. A significant reduction in the number of [^{3}H] imipramine binding sites was observed in non-medicated patients diagnosed as unipolar depressed compared with age and sex matched controls.

Studies with @-carbolines were initiated to determine if these compounds, which inhibit [^{3}H] diazepam binding with high affinity *in vitro*, possess a pharmacologic action. It was observed that 3-hydroxymethyl-@-carboline, which inhibits [^{3}H] diazepam binding *in vitro* (K_i of 1470 nM) antagonized both the anticonvulsant and anxiolytic actions of diazepam in mice at doses which do not elicit gross behavioral alterations (including sedation) in these animals.

This compound also antagonized the anxiolytic actions (in mice) of both meprobamate and pentobarbital. Other β-carbolines (e.g. 3-formyl-β-carboline) were also found to antagonize the pharmacologic actions of diazepam. The structure activity relationships of a series of β-carbolines revealed the importance of substitution in the 3 position for optimum affinity at the benzodiazepine receptor. The molecules in this series with the highest affinities for the benzodiazepine receptor all possessed a carbonyl oxygen at the 3 position. The length of the carbon substitution at this position did not significantly change affinity. Substitution at the C-1 position resulted in a dramatic loss in activity, as did reduction of the pyridine ring to yield the isosteric tetrahydro-β-carboline series. Several of the β-carbolines examined had Hill coefficients significantly less than unity for displacement of [^{3}H] diazepam, suggesting a preferential interaction with one subpopulation of benzodiazepine receptor. These data also support the hypothesis that the tremors and sedation observed with many β-carbolines may be unrelated to their interaction with benzodiazepine receptors.

Barbiturates were found to have a dual action in enhancing the binding of [^{3}H] benzodiazepines to benzodiazepine receptors in the CNS. Using pentobarbital as a model barbiturate, it was found that relatively high concentrations (corresponding to brain levels achieved after anesthetic doses) enhance [^{3}H] diazepam binding by increasing receptor affinity. At approximately 10-fold lower concentrations (corresponding to tissue levels achieved after anxiolytic doses), pentobarbital potentiated the GABA-enhanced binding of [^{3}H] diazepam. At maximally effective concentrations of GABA, addition of pentobarbital resulted in no more than an additive action. The "direct" actions of pentobarbital were anion dependent (both the potency and maximum effect were lowered in the absence of chloride, iodide, bromide or other ions which readily penetrate chloride channels) and blocked by the GABA-antagonist, bicuculline or the chloride ionophore blocker, picrotoxin. The "direct" (i.e. in the absence of exogenously added GABA) actions of pentobarbital were also sensitive to physical manipulation of the tissue, since freeze-thawing and detergent treatment markedly reduced this effect to increase [^{3}H] diazepam binding. A large number of barbiturates share this action with pentobarbital, such as hexobarbital, amobarbital, and the convulsant barbiturate dimethylbutylbarbituric acid (DMBB). Furthermore, marked regional differences were observed in rodent brain with respect to both the potency and efficacy of barbiturates in enhancing benzodiazepine receptor affinity. The phenomenon of a dual enhancement of benzodiazepine receptor affinity by barbiturates is reminiscent of the electrophysiological actions of barbiturates observed on cultured spinal cord neurons. That is, at relatively low concentrations of barbiturate, a potentiation of the depressant actions of GABA are observed, while at higher concentrations, a direct depressant action on neuronal excitability is induced by barbiturates such as pentobarbital. These observations have been instrumental in constructing a model of the benzodiazepine receptor as one component of a functionally linked supramolecular complex consisting of a benzodiazepine receptor, GABA receptor, and chloride ionophore which may mediate the pharmacologic actions of a variety of drugs including barbiturates, benzodiazepines, triazolopyridazines and pyrazolopyridines.

Studies with a brain slice preparation from rat cerebral cortex suggest that this system may be an excellent model for examining the in vitro regulation of benzodiazepine receptors. High affinity, saturable, and stereospecific binding of [^{3}H] flunitrazepam has been observed in brain slices from a variety of brain regions. Kinetic parameters (such as B_{max} and K_d) approximate those found in disrupted cell preparations. This system may be superior to isolated membrane preparations in certain studies requiring the intact cellular architecture of the brain.

Brain extracts of rats which have been sleep deprived for 24 hours have been observed to contain endogenous compounds which enhance [^{3}H] diazepam binding more than identical extracts prepared from sleep deprived controls. This compound(s) is dialyzable and heat stable. In addition, less of an inhibitory factor may also be produced through sleep deprivation. Further studies are now in progress to characterize these factors.

Several N^6-substituted adenosine derivatives such as KM-12, (2'-chlorobenzyl)-N^6-[2-(4-chlorophenyl)-bicyclo-2.2.2.octyl-(3)]adenosine, have been demonstrated to affect the benzodiazepine receptor either by competitive inhibition of the receptor (KM-12) or by increasing benzodiazepine receptor number (KM-13). Further more, both KM-12 and KM-13 possess marked sedative and anticonvulsant actions, and KM-13 has been demonstrated to have an anxiolytic action with a potency comparable to chlordiazepoxide (Librium). Recently, it has been demonstrated that other N^6-substituted adenosines such as cyclohexyladenosine (CHA) and L-phenylisopropyladenosine are potent long lasting sedatives when administered to mice. These compounds are not potent inhibitors of [^{3}H] diazepam binding but have high affinities for adenosine (A_1) receptors in the CNS. We have investigated the potency of KM-12, KM-13 and other related N^6-substituted adenosine derivatives at A_1 receptors. Both KM-12 and KM-13 inhibit [^{3}H] CHA binding with affinities (K_i) of less than 100 nM. These data suggest that the sedative/hypnotic actions of some N^6-substituted adenosine derivatives may be related to their interaction with adenosine rather than benzodiazepine receptors. In conjunction with these findings, we have demonstrated that administration of an inhibitor of adenosine deaminase, 9-(erthryo-hydroxynonyl)adenine (EHNA), which prevents the metabolism of endogenous adenosine, results in a long lasting sedative action in mice. The time course of the sedation appears to correlate with the inhibition of this enzyme.

The binding of biologically active ^{125}[I] cholecystokinin (CCK) to rat brain membranes was established. This binding was of high affinity, saturable, reversible and inhibited by biologically-active cholecystokinin peptides. Kinetic and competition studies suggest that these high affinity binding sites represent physiologically relevant receptors for CCK in brain. Characterization of the cellular elements in which CCK receptors are concentrated in the basal ganglia was carried out by a series of chemical and physical lesion studies. These experiments revealed that CCK receptors are highly localized to intrinsic neuronal elements in the caudate nucleus with at least 75% of the receptors on neuronal cell bodies. Postmortem studies of the brain areas from patients with Huntington's chorea confirmed the marked localization of CCK receptors to neurons of the basal ganglia since a marked decrease was observed in the brains of Huntington's patients when compared to age-matched controls. More recent

studies have also demonstrated marked decreases in the density of CCK receptors in affected areas of cerebral cortex in patients dying of both Huntington's and Alzheimer's disease. The latter findings suggest that CCK receptors may be involved in higher cortical (cognitive) functions.

Dr. Richard Hauger studied [^{3}H] ouabain binding to brain membranes. [^{3}H] ouabain binds to two distinct "high affinity" sites in various areas of rat brain; whereas human brain and erythrocyte ghosts only have one high affinity site. In the rat striatum "high affinity" 17.4 and 400 nM and binding capacities of 12.0 and 36.0 pmol/mg, respectively. Human erythrocyte oubain receptors exhibited an apparent K_D of 9.6 nM and binding capacity of 0.94 pmol/mg . In contrast "high affinity" sites were absent from peripheral tissues i.e. heart and kidney. Following lesions of the rat striatum with kainic acid, a marked decrease (40-60%) of both "high affinity" ouabain binding sites was observed. These results suggest that both binding sites are localized to the neuronal Na^+-K^+ ATPase. This hypothesis was further supported by studies on postmortem brain samples from patients with Huntington's disease where a marked decrease in [^{3}H] ouabain binding was observed in the caudate nucleus when compared to matched controls. Current studies are directed toward understanding the physiological importance of "high affinity" ouabain binding sites in neuronal function.

As an extension of our studies benzodiazepines receptor interactions Dr. Richard Ross studied the effects of two benzodiazepines, flurazepam and diazepam, on the firing rates of neurons in the pars reticulata of the substantia nigra. These studies were made possible by the collaborative support of Dr. Judith Walters of the NINCDS. The pars reticulata region receives a considerable GABAergic input, and its neurons are known to be inhibited by iontophoretically applied GABA and systemically administered muscimol, a GABA agonist. It was hypothesized that the benzodiazepines, which potentiate the action of GABA in other physiological systems, would exert an inhibitory effect on the firing rates of extracellularly recorded pars reticulata cells. It was found that i.v. flurazepam produced a modest inhibition and that i.v. diazepam had a more prominent inhibitory effect. Picrotoxin, an antagonist at the GABA related ionophore, reversed the inhibition. Studies underway are designed to investigate the actions of putative endogenous ligands for the benzodiazepine receptor in this system and to explore the possibility that the methylxanthines, including caffeine, may antagonize the physiological effects of the benzodiazepines. In addition, we plan to use the pressure ejection technique to study the effects of locally applied benzodiazepines on pars reticulata neurons.

Significance to Biomedical Research and the Program of the Institute: Benzodiazepines and tricyclic antidepressants are among the most widely prescribed drugs in current therapeutic use. An understanding of the mechanism of action of these compounds, as well as the identification of endogenous compounds which physiologically control these sites could be of fundamental importance to our understanding of a number of neuropsychiatric disorders including epilepsy, anxiety-neuroses, sleep disorders, alcoholism, and

depression. Furthermore, these studies should provide information useful in the development of more effective therapeutic agents which may lack deleterious side effects.

Proposed Course of Project: The significance of the benzodiazepine-GABA receptor-chloride ionophore receptor complex will be examined in behavioral states where altered sensitivity to centrally acting drugs has been demonstrated. The interaction of benzodiazepine and adenosine receptors will be investigated by studying behavioral modification of animals (e.g sleep deprivation, genetic breeding) as well as further study of the structural requirements for the interaction of agonists and antagonists with each of these recognition sites. The heterogeneity of benzodiazepine receptors will be further explored by the use of β-carbolines which have a preferential interaction with one receptor subtype. The brain slice preparation will be used to examine in vitro regulation of benzodiazepine and GABA receptors. Purification of the "serotonin transporter" in human platelet and brain membranes will be attempted by the use of irreversible ligands (i.e. nitroimipramine derivatives) to these sites. Since postmortem studies of norepinephrine and serotonin uptake have not been possible because of the postmortem lability of the uptake processes, studies of [^{3}H] tricyclic antidepressant binding to these uptake sites in postmortem brain specimens from psychiatrically ill patients will be carried out. Further characterization and purification of the imipramine binding site and elucidation of its relationship to other proteins involved in the energy-dependent uptake of serotonin across the platelet and neuronal membrane (e.g. $Na+K^+$ ATPase) will be attempted. Clinical studies in depressed patients, anorexic, and schizophrenic patients will eventually compare the purified protein from platelet membranes in an attempt to sort out the observed decreases in this protein among depressed patients.

Miscellaneous: Patents: US Patent No. 4,239,744, A radioreceptor assay for benzodiazepines in plasma and other biological fluids awarded December, 1980.

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Publications:

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Note: Z01 MH 01836-03 CP is a combination of Z01 MH 01838-02 Studies on the Interaction(s) of Peripheral Hormones with CNS Neurotransmitter Receptors and Z01 MH 01836-03 Studies of CNS Neurotransmitter and Drug Receptors.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01838-01 CP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies on the Interaction(s) of Peripheral Hormones with CNS Neurotransmitter Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Note: Z01 MH 01838-01 CP has been incorporated into Z01 MH 01836-03 CP

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00422-10 LCS

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neuropharmacology of Circadian Rhythms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Circadian rhythms and environmental lighting regulate a number of endocrine and behavioral functions. The rat pineal gland provides an ideal model for the study of mechanisms which generate and control circadian rhythms. Evidence indicates that the neurotransmitter acetylcholine is involved in the central regulation of circadian rhythms by light. There is a circadian rhythm in the rat pineal in levels of the synaptic protein designated protein I, which is mediated by β-adrenergic neurotransmission.

Project Description:

Objectives: To elucidate the neuropharmacology of circadian rhythms.

Methods Employed: Biochemical, pharmacologic, surgical, immunologic, radioactive trace techniques.

Major Findings: Administration of the cholinergic agonist, carbachol, into the cerebral ventricles, or near the suprachiasmatic nuclei of the hypothalamus, mimics the effects of light on pineal and other circadian rhythms. This effect is mediated by nicotinic cholinergic receptors. Infusion of α-bungarotoxin, a specific and irreversible nicotinic blocker, prevents the effects of light on the rat pineal. These and other results implicate the neurotransmitter acetylcholine in the regulation of the circadian pacemaker by light.

Levels of the synaptic protein designated protein I, a substrate for cyclic AMP-dependent protein kinase which is phosphorylated after synaptic transmission, are higher at night than during the day in the rat pineal. The nocturnal rise reflects a true circadian rhythm, is blocked by light or injection of propranolol, and does not occur in animals whose pineals have been decentralized or denervated. The nocturnal increase can be mimicked by the β-adrenergic agonist isoproterenol in vivo or by isoproterenol or cyclic AMP in vitro; thus, the increase in the level of protein I is a consequence of nocturnal β-adrenergic stimulation and may participate in the sequence of events leading to the circadian rise in pineal indoleamine metabolism.

Significance to Biomedical Research: Circadian rhythms occur in hormone levels, activity, mood, temperature, and other physiologic functions. Elucidation of the mechanisms generating or regulating circadian rhythms are of broad clinical and biologic interest. Similarly, the consequences of neurotransmission and the mechanisms by which neurotransmitter-receptor interactions are transduced into physiological changes are important for the understanding of hormone or neurotransmitter actions.

Proposed Course of Project: Mechanisms generating and regulating circadian rhythms and the effects of light will be explored further.

Publications:

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Skolnick, P., Paul, S.M., Zatz, M., and Eskay, P.: "Brain-specific" benzodiazepine receptors are localized in the inner plexiform layer of rat retina. Eur. J. Pharm. 66: 133-136, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00425-05 LCS

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Peripheral and Central Catecholamines in Hypertension and Stress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH ADAMHA NIH, Bethesda, Maryland, 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	6.0	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to study the role of catecholamines in the central and peripheral system in experimental and genetic hypertension and stress, and has now been extended to study the participation of other biogenic amines and estrogens in the central regulation of neurovegetative functions.

Central catecholamines and histamine are involved in the regulation of all forms of hypertension studied, and in stress. Adrenaline is formed in brain by two enzymes with different localization and substrate specificity. Adrenaline may regulate noradrenaline metabolism in the pineal gland. There is a circadian rhythm for catecholamines in posterior pituitary. Estrogens modulate catecholamine and MAO activity in pituitary gland and brain stem.

There are alterations in kidney levels of catecholamines and in α-receptor number in genetic hypertension. Genetically hypertensive rats also present alterations in heart catecholamines, in selective increases in catecholamine formation in the celiac and stellate ganglia.

Continuation

Names, Laboratory and Institute Affiliations, and Titles of Principal Investigators and All Other Professional Personnel Engaged on the Project

Others (continued): C. Ferrario, Head, Hypertension Section, Cleveland Clinic, Ohio
J. Iwai, Section Head, Brookhaven National Laboratories, New York
W. Pettinger, Professor, Department of Pharmacology, Southwestern University, Texas
D. Reis, Professor, Department of Neurology, Cornell University, New York

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Project Description:

Objectives: To study the role of central and peripheral catecholamines and other biogenic amines in hypertension and stress.

Methods Employed: Anatomical: microdissection of brain nuclei. Surgical: lesions of brain nuclei by specific neurotoxins and knife cuts. Surgical baroreceptor deafferentation. Biochemical: enzymatic-isotopic micromethods for the measurement of biogenic animes, their metabolites, and related enzymes.

Major Findings:

Brain adrenergic neurons - Brain stem adrenaline neurons in DAHL salt-sensitive, genetically hypertensive rats show increased activity of the adrenaline-forming enzyme, PNMT.

PNMT increases in brain stem but decreases in forebrain during stress. Complete hemisections at the superior colliculus level do not produce changes in PNMT activity in forebrain areas, but resulted in a 50% decrease in forebrain noradrenaline, indicating the absence of a direct PNMT-containing projection from brain stem to forebrain areas, and suggesting that two different methyltransferases could be involved in the formation of adrenaline in brain.

Brain catecholamines in stress - Specific changes in catecholamine levels occur in preoptic and septal nuclei during acute stress.

Pineal catecholamines - Adrenaline is present in rat pineal, and its levels show a circadian rhythm synchronic to that of other catecholamines. The major source of pineal adrenaline is the adrenal medulla, although the gland contains PNMT activity, localized postsynaptically. Pineal adrenaline levels increase several-fold after stress and may regulate noradrenaline metabolism in the pineal.

Posterior pituitary catecholamines - Adrenaline, together with noradrenaline and dopamine, is present in the posterior pituitary gland. All catecholamines have a synchronic circadian rhythm in the pituitary gland.

Peripheral catecholamines in hypertension - In the kidney of DAHL salt-sensitive hypertensive rats, there are discrete changes in catecholamine levels in specific cortical and medullary areas, and an increase in α-adrenergic receptors.

There is a selective increase in stellate ganglia and celiac ganglia catecholamine levels in spontaneously hypertensive rats.

In the heart, there are no changes in β-adrenergic receptors in DAHL hypertensive or in SH rats. In SHR, there are no changes in catecholamine levels, but there is an increased turnover of catecholamines in DAHL rats. Increased methylation of chloroform-methanol extractable material (not phospholipids) occurs in heart membranes after stress and propranolol treatment.

Circadian rhythms in the brain - There are circadian rhythms for MAO type A and type B and catecholamines in specific brain stem areas.

Interrelation between estradiol and catecholamines - In ovariectomized rats, estradiol treatment decreases type A and increases type B MAO in brain stem nuclei; produces no changes in pineal or brain stem catecholamines, and a decrease in dopamine and noradrenaline levels in posterior pituitary gland.

Brain histamine in SHR - A selective increase in histamine in individual hypothalamic nuclei is present in young and adult SHR.

Significance to Biomedical Research: The studies on biogenic amines and the central regulation of blood pressure and stress could help to explain some aspects of the role of specific brain structures in neuroendocrine functions, and also to further explain some basic aspects of the physiology of these brain transmitters.

Specific drugs which interfere with the neurotransmitter metabolism could be developed for application to the therapy of neuroendocrine disorders. As an example, inhibitors of adrenaline synthesis can lower blood pressure and are now being studied for their possible application to human pathological states.

Proposed Course of Project: Further studies will be conducted to determine the characteristics of the adrenaline-forming enzymes in brain, including specific lesions and studies with specific PNMT antibodies.

The role of catecholamines, serotonin and histamine in genetic hypertension and stress will be studied with emphasis in the determination of amine metabolites, and will be focused in brain stem, hypothalamic and limbic system areas.

The role of pineal adrenaline and posterior pituitary catecholamines will be studied after modification of the conditions regulating their circadian rhythms.

Further studies will be performed to analyze the nature of the methylated compounds formed by cardiac membranes when incubated with SAMe, during stress and propranolol treatment.

Manipulations of the sodium diet in susceptible animals will be performed to further characterize the observed peripheral (heart, pineal, pituitary and kidney) and central changes in biogenic amines.

Publications:

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Saavedra, J.M., and Torda, T.: Increased brain stem and decreased hypothalamic adrenaline-forming enzyme after acute and repeated immobilization stress in the rat. Neuroendocrinology 31: 142-146, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00426-05 LCS

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Catecholestrogens: Synthesis and Developmental Aspects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Hoffman, Research Associate	LCS NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH
Others:	M. Poth, Clinical Associate	IRP NICHHD
	S. Paul, Chief, Unit on Preclinical Pharmacology	CPB NIMH

COOPERATING UNITS (if any)
Unit on Preclinical Pharmacology, Clinical Psychiatry Branch, NIMH
Neonatal and Pediatric Medicine Branch, IRP/NICHHD

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Section on Pharmacology

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NIMH ADAMHA NIH, Bethesda, Maryland, 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00427-04 LCS

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Phospholipid Methylation and Signal Transduction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. Hirata, Visiting Scientist	LCS NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH
	F. Crews, Research Associate	LCS NIMH
	D. Bareis, Research Associate	LCS NIMH
	N. Iwata, Guest Worker	LCS NIMH
	A. Hotchkiss, Research Associate	LCS NIMH
Others:	T. Hoffman, Senior Scientist	LID NCI
	Y. Ito, Visiting Scientist	ORDA NIAID
	M. Nirenberg, Chief, Lab. of Biochemical Genetics	LBG NHLBI
	R. Siraganian, Chief, Section on Clinical Immunology	LMI NIDR
	M. Vaughan, Chief, Laboratory of Cellular Metabolism	LCM NHLBI
	K. Ishizaka, Professor of Immunology, Johns Hopkins University	

COOPERATING UNITS (if any)
LID/NCI; LBG and LCM/NHLBI; LDBA and LMI/NIDR; ORDA/NIAID;
Division of Immunology, Johns Hopkins University, Baltimore, Maryland;
Department of Biochemistry, New York State University, New York

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NIMH ADAMHA NIH, Bethesda, Maryland, 20205

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5.0	5.0	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Phospholipid methylation cascade was found to be associated with receptor mediated transmission of biological signals in several systems. The phospholipid cascade involves interaction of cell surface membrane receptors with specific ligands, activation of phospholipid methylation, influx of Ca^{2+}, activation of phospholipase A_2, liberation of arachidonic acid and lysophosphatidyl choline. Among the various cellular processes utilizing this cascade are IgE mediated release of histamine from mast cells and leukemic basophils, stimulation of chemotaxis in neutrophils by fMet-Leu-Phe, destruction of malignant tumors by natural killer cells and the generation of prostaglandin by bradykinin stimulated fibroblasts. A phospholipase inhibiting protein, lipomodulin, was purified almost to homogeneity and its properties studied. An antibody to lipomodulin was found in the sera of subjects with rheumatic diseases.

Continuation

Names, Laboratory and Institute Affiliations, and Titles of Principal Investigators and All Other Professional Personnel Engaged on the Project

Others (continued): V. Ventor, Assistant Professor of Biochemistry, New York State University
E. Schiffmann, Research Chemist LDBA NIDR
A. McGivney, Research Associate LMI NIDR

Project Description:

Objectives: To study the role of phospholipid methylation in the transduction of neurotransmitter, hormone, drug and antibody signals across cell membranes.

Methods Employed: Enzymatic, radiometric, immunological, and pharmacological techniques.

Major Findings: We have previously shown that stimulation of mast cells rat basophilic leukemia (RBL) by an antibody to the IgE receptor or IgE antisera causes a transient increase of phospholipid methylation, an influx of Ca^{2+}, activation of phospholipase A_2 and release of histamine. Work done in the past year has demonstrated that the IgE receptor is linked to both phospholipid methyltransferase and adenylate cyclase. The methyltransferase activation serves as the "on" signal for histamine release and activation of adenylate cyclase serves as the "off" signal. Additional evidence for the role of phospholipid methylation in the influx of Ca^{2+} and the release of histamine was obtained by producing variants of RBL cells by chemical mutagens. Two mutants were found; one which lacks phospholipid methyltransferase I (PMT I) and the other which is deficient in phospholipid methyltransferase II (PMT II). Neither of these variant RBL cells allow an influx of Ca^{2+}, or release of histamine when stimulated with RBL IgE antigens. Upon fusing these two cells deficient in methyltransferase enzymes, the ability of the hybrids to synthesize methylated phospholipids, to take up Ca^{2+} and release histamine was restored.

Stimulation of rabbit neutrophils with the chemotactic peptide fMet-Leu-Phe caused an increased turnover of methylated phospholipids, an influx of Ca^{2+} and activation of phospholipase A_2 as measured by the release of arachidonic acid. Inhibition of phospholipid methylation blocked Ca^{2+} influx, arachidonic acid release and chemotaxis.

When stimulated with bradykinin, human fibroblasts generate cyclic AMP. Bradykinin also was found to stimulate phospholipid methylation, Ca^{2+} influx, release of arachidonic acid, formation of prostaglandin E and elevation of cyclic AMP. Inhibition of phospholipase A_2 blocks prostaglandin release and cyclic AMP elevation. Inhibition of phospholipid methylation partially blocks Ca^{2+} influx, phospholipase A_2, and activation of prostaglandin release but had no effect on bradykinin stimulated cyclic AMP elevation.

β-Adrenergic receptor isolated from turkey erythrocytes could be inserted into human erythrocyte membranes which lack these receptors. The insertion of the β-adrenergic receptors into human erythrocyte membranes markedly repressed phospholipid methyltransferase. Binding of β-adrenergic ligands to human red cell β-adrenergic receptors overcame the inhibition of phospholipid methyltransferase.

Lipomodulin, a phospholipase inhibitory protein, was purified from conditioned media of neutrophils which were treated with glucocorticoids. This protein was purified almost to homogeneity and was found to have a molecular weight of about 40,000 daltons. When lipomodulin was phosphorylated, it no longer had inhibitory action towards phospholipases. Sera of patients with rheumatic diseases were found to have an auto-antibody to the phospholipase inhibitory protein lipomodulin.

Stimulation of isolated chromaffin cells with acetylcholine, nicotine or veratridine caused a release of catecholamines and [^{14}C]-arachidonic acid previously incorporated into phospholipids. Nicotinic receptor blocking agents, hexamethonium or curare, inhibited arachidonic acid and catecholamine release. These findings suggest that arachidonic acid may play a role in the receptor mediated release of catecholamines.

Significance to Biomedical Research: The phospholipid methylation cascade appears to be a fundamental mechanism of many cell functions and should have implication in allergy, rheumatic disease, and generation of malignancy in cells.

Proposed Course of Project: Purification and characterization of methyltransferases will be continued. Properties of antibodies for β-adrenergic receptors, methyltransferase and phospholipase A_2 inhibitor protein(s) will be characterized and their antibodies should be applied for the survey of their normal biological functions and their role in rheumatic diseases. Role of phospholipid methylation in nerve function will be explored.

Publications:

Axelrod, J., and Hirata, F.: Lipids and the transduction of biological signals through membranes. In Brambilla, F., Racagni, G., and de Wield, D. (Eds.): Progress in Psychoneuroendocrinology. Elsevier/North Holland Press, Inc., 1980, pp. 1-12.

Crews, F.T., Hirata, F., and Axelrod, J.: Identification and properties of methyltransferases that synthesize phosphatidylcholine in rat brain synaptosomes. J. Neurochem. 34: 1491-1498, 1980.

Crews, F.T., Hirata, F., and Axelrod, J.: Phospholipid methyltransferase asymmetry in synaptosomal membranes. Neurochem. Res. 5: 983-991, 1980.

Hirata, F.: The regulation of lipomodulin, a phospholipase inhibitory protein, in rabbit neutrophils by phosphorylation. J. Biol. Chem., in press.

Hirata, F., and Axelrod, J.: Phospholipid methylation and biological signal transmission. Science 209: 1082-1090, 1980.

Hirata, F., del Carmine, R., Nelson, C.A., Axelrod, J., Schiffmann, E., Warabi, A., De Blas, A.L., Nirenberg, M., Manganiello, V., Vaughan, M., Kumagai, S., Green, I., Decker, J.L., and Steinberg, A.D.: Presence of autoantibody for phospholipase inhibitory protein, lipomodulin, in patients with rheumatic diseases. Proc. Natl. Acad. Sci. USA 78: 3190-3194, 1981.

Hirata, F., Schiffmann, E., Venkatasubramanian, K., Salomon, D., and Axelrod, J.: A phospholipase A_2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. Proc. Natl. Acad. Sci. USA 77: 2533-2536, 1980.

Hoffman, T., Hirata, F., Bougnoux, P., Fraser, B.A., Goldfarb, R.H., Herberman, R.B., and Axelrod, J.: Phospholipid methylation and phospholipase A_2 activation in cytotoxicity by human natural killer cells. Proc. Natl. Acad. Sci. USA, in press.

Mallorga, P., Tallman, J.F., Henneberry, R.C., Hirata, F., Strittmatter, W.T., and Axelrod, J.: Mepacrine blocks beta-adrenergic agonist-induced desensitization in astrocytoma cells. Proc. Natl. Acad. Sci. USA 77: 1341-1345, 1980.

Tallman, J.F., Gallager, D.W., Mallorga, P., Thomas, J.W., Strittmatter, W., Hirata, F., and Axelrod, J.: Studies on benzodiazepine receptors. In Pepeu, G., Kuhar, M.J., and Enna, S.J. (Eds.): Receptors for Neurotransmitters and Peptide Hormones. New York, Raven Press, 1980, pp. 277-283.

Torda, T., Yamaguchi, I., Hirata, F., Kopin, I.J., and Axelrod, J.: Mepacrine treatment prevents immobilization-induced desensitization of beta-adrenergic receptors in rat hypothalamus and brain stem. Brain Res. 205: 441-444, 1981.

Venkatasubramanian, K., Hirata, F., Gagnon, C., Corcoran, B.A., O'Dea, R.F., Axelrod, J., and Schiffmann, E.: Protein methylesterase and leukocyte chemotaxis. Mol. Immunol. 17: 201-207, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00428-02 LCS

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Protein Carboxyl-Methylation: A Post Translational Modifier of Protein Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Y. Kloog, Fogarty International Fellow — LCS NIMH
J. Axelrod, Chief, Section on Pharmacology — LCS NIMH
A. Hoffman, Research Associate — LCS NIMH

Others: D. Flynn, Dept. of Pharmacology, University of Miami

COOPERATING UNITS (if any)
Department of Pharmacology, School of Medicine, University of Miami, Miami, Florida

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Section on Pharmacology

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	2.0	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Enzymatic carboxymethylation of proteins is a post-translational modification. By changing the structure and properties of membrane proteins, carboxymethylation provides an important regulatory mechanism. Receptors being the molecules that receive signals from the exterior of the cell and transmit information to its interior are thus obvious targets for regulating modification.

The purified acetylcholine receptor (AcChoR) is a complex of five polypeptides and is an excellent substrate for the enzyme protein carboxyl methylase (PCM). AcChoR enriched membranes are also excellent substrates for PCM. These membranes are closed right-side out vesicles. By the use of freeze-thaw techniques, PCM can be introduced into the vesicles and methylate them from the inside. Separation of the methylated pure AcChoR, and inside methylated vesicles on gel electrophoresis shows that all of the receptor subunits are methylated. The 60K polypeptide has the highest specific activity, while the 40K the lowest. Thus the data shows both specificity of the receptor subunits

methylation and that all face the inside of the AcChoR enriched vesicles.

Project Description:

Objectives: To learn which of the methylated AcChoR subunits is affected upon stimulation with cholinergic drugs, and to determine how their activity is altered.

Methods Employed: Enzymatic, pharmacologic.

Major Findings: AcChoR is an excellent substrate for PCM. It can be methylated in its purified form, in detergent solubilized membranes, in membranes, and from the inside of the AcChoR enriched vesicles. All of the receptor subunits are methylated from the inside of the vesicles, suggesting they face the interior side of the post synaptic membrane.

Significance to Biomedical Research: Carboxymethylation may prove to be a key regulatory step in the activity of a number of enzymes and receptors by reducing negative charges on membrane proteins.

Proposed Course of Project: The precise location of methylation of the ACho receptor will be delineated and the functional consequences of methylation will be investigated.

Publications:

Kloog, Y., Flynn, D., Hoffman, A.R., and Axelrod, J.: Enzymatic carboxymethylation of the nicotinic acetylcholine receptor. Biochem. Biophys. Res. Commun. 97: 1474-1480, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00429-02 LCS

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biosynthesis of Nonpolar Methylated Lipids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Zatz, Medical Officer (Research)	LCS NIMH
Others:	P. Dudley, Staff Fellow	LVR NEI
	S. Markey, Pharmacologist	LCS NIMH
	Y. Kloog, PHS International Fellow	LCS NIMH

COOPERATING UNITS (if any)

Laboratory of Vision Research, NEI

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Laboratory of Clinical Science

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NIMH ADAMHA NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several tissues incorporate methyl groups from S-adenosylmethionine into nonpolar lipids. These products include fatty acid methyl esters, ubiquinone, and methylated thiols. The enzymatic methylation of fatty acids occurs in cell membranes, including plasma membranes, and is prominent in secretory organs such as lung and parotid.

Project Description:

Objectives: To identify the nonpolar lipids formed from S-adenosylmethionine and to investigate their biochemistry and physiology.

Methods Employed: Biochemical, chromatographic, chemical, pharmacologic, and radioactive trace techniques.

Major Findings: Incubation of membranes from lung, retina, salivary gland, or rat leukemic basophils with radioactive S-adenosylmethionine (SAM) results in the formation of nonpolar methylated lipids. The major products were identified as fatty acid methyl esters by mass spectrometry after isolation by extraction, column chromatography, HPLC, and GC. Fatty acid methylation occurs via an enzymatic process. The reaction occurs in microsomal membranes and plasma membranes.

The major nonpolar methylated lipid formed in growing rat basophilic leukemia cells incubated with radioactive methionine was identified as ubiquinone-9. Small quantities of fatty acid methyl esters were also found. Another product formed was [2-methylthio]-benzothiazole. This was identified as a product of the known enzyme S-methyltransferase acting on the thiol substrate which was present as a contaminant of the enriched media. Interference by thiol methylation can be avoided by the use of simple buffers and thorough drying of organic extracts.

Significance to Biomedical Research: The role of membrane lipids in cellular function, particularly in signal transduction, is currently being recognized and elucidated. Methylation of fatty acids by plasma membranes and secretory tissues suggests that the reaction may play a dynamic role in cellular function.

Proposed Course of Project: The biochemistry and physiology of fatty acid methylation will be investigated.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00430-02 LCS

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Tricyclic Antidepressants, Alpha-adrenergic Blocking Agents and Response of Beta-adrenergic Receptor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: F. Crews, Research Associate — LCS NIMH

Others: S. Paul, Chief, Unit on Preclinical Pharmacology — CPB NIMH

COOPERATING UNITS (if any)
Unit on Preclinical Pharmacology, Clinical Psychiatry Branch, NIMH

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NIMH ADAMHA NIH, Bethesda, Maryland, 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

TERMINATED

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00431-02 LCS

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Lipid Metabolism, Membrane Viscosity and Alcohol Dependence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. Crews, Research Associate	LCS NIMH
	F. Hirata, Visiting Scientist	LCS NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH
Others:	E. Majchrowitz, Research Biologist	LPS NIAAA

COOPERATING UNITS (if any)

Laboratory of Preclinical Studies, NIAAA

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NIMH ADAMHA NIH, Bethesda, Maryland, 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

TERMINATED

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00432-02 LCS

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Protein Carboxyl-methylation: A Post Translational Modifier of Protein Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Hoffman, Research Associate	LCS NIMH
	Y. Kloog, PHS International Fellow	LCS NIMH
	A. Hotchkiss, Research Associate	LCS NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH
Others:	C. Gagnon, Faculty of Medicine, Laval University, Quebec, Canada	

COOPERATING UNITS (if any)
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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

TERMINATED

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00433-01 LCS

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Role of Neuropeptides in Neuroendocrine Regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. M. Saavedra, Medical Officer (Psychiatry) LCS NIMH

Others:
C. Chevillard, Guest Worker LCS NIMH
J. Fernandez-Pardal, Guest Worker LCS NIMH
F. Correa, Guest Worker LCS NIMH
Y. Kloog, PHS International Fellow LCS NIMH
F. Dray, Head, Unit on RIA, Institute Pasteur, France
C. Rougeot, Research Assistant, Unit on RIA, Institute Pasteur, France
K. Gerozitssis, Established Investigator, Unit on RIA, Institute Pasteur, France
G. Pelletier, Professor, Department of Molecular Endocrinology, Laval University, Canada
A. Negro-Vilar, Assoc. Prof., Dept. of Psysiol., Southwestern Univ., TX.

COOPERATING UNITS (if any)
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Department of Molecular Endocrinology, Laval University, Canada
Department of Physiology, Southwestern University, Texas

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NIMH ADAMHA NIH, Bethesda, Maryland, 20205

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5.0	5.0	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to study the role of brain neuropeptides in genetic hypertension and stress and has now been extended to study the interrelationship between different neuropeptides and their role in neuroendocrine regulation.

Genetically hypertensive rats show changes in vasopressin and somatostatin metabolism when submitted to stress, and a naloxone-reversible and propranolol-reversible decrease in pain sensitivity.

Angiotensin-converting enzyme (ACE) activity is heterogeneously distributed in the CNS. There are changes in hypothalamic and posterior pituitary ACE activity in rats lacking vasopressin, changes in which are reversible after vasopressin replacement.

Project Description:

Objectives: To study the functions of central neuropeptides and their role in neuroendocrine regulation.

Methods Employed: Anatomical: microdissection of brain nuclei and specific lesions by knife cuts; immunohistofluorescent techniques. Biochemical: radioimmunoassays of neuropeptides; high pressure liquid chromatography.

Major Findings:

Endogenous opiates - Young and adult spontaneously hypertensive rats (SHR) show a naloxone-reversible and propranolol-reversible decrease in pain sensitivity. Endogenous opiates might be related to brain catecholamines, and are modified in SHR.

Neuroanatomy of peptidergic systems in brain - ACTH, β-LPH and α-MSH containing cell bodies are localized in the area of the arcuate nucleus. Their projections were studied by selective knife cuts or neonatal injections of selective neurotoxins.

Vasopressin and somatostatin in genetic hypertension - SHR have less vasopressin than normotensive Wistar Kyoto rats in the supraoptic nucleus. When stressed, SHR increase their vasopressin levels, whereas normotensive animals do not.

After stress, levels of somatostatin decrease in normotensive rats, but fail to do so in SHR.

Central angiotensin system - A sixty-fold difference exists in selective brain areas of the rat regarding its angiotensin-converting enzyme activity. In selective hypothalamic nuclei, and in the posterior pituitary, rats lacking vasopressin (Brattleboro rats) show vasopressin-reversible changes in angiotensin enzyme activity.

Neuropeptides in hypertension - ACTH, LHRH, substance P, β-endorphin, m-enkephalin and α-MSH have been studied in selective brain areas from rats with spontaneously (genetic) hypertension. There are changes in substance P and ACTH.

Proposed Course of Project: We plan to study further the physiological and pathophysiological interactions between the vasopressin and angiotensin systems in brain and pituitary gland.

The role of vasopressin and somatostatin in SHR will be studied by a combination of lesions, the use of antibodies, drug treatments and physiological manipulations.

Significance to Biomedical Research: The study of the interactions between different neuropeptides and between neuropeptides and catecholamines will clarify part of the role of these compounds in the regulation of blood pressure and other related neuroendocrine functions. The use of drugs which interfere with the metabolism and function of neuropeptides could result in future therapeutic advantages in the treatment of neuroendocrine disorders.

Publications:

Saavedra, J.M.: Naloxone reversible decrease in pain sensitivity in young and adult spontaneously hypertensive rats. Brain Res. 209: 245-249, 1981.

Saavedra, J.M.: Biogenic amines and neuropeptides play a role in the central regulation of genetic hypertension. In Worcel, M., Bonvalet, J.P., Langer, S.Z., Menard, J., and Sassard, J. (Eds.): New Trends in Arterial Hypertension, INSERM Symp. 17. Elsevier/North-Holland Biomedical Press, 1981.

Pelletier, G., Leclerc, R., Saavedra, J.M., Brownstein, M.J., Laudry, H., Ferland, L., and Labrie, F.: Distribution of β-lipotropin (β-LPH), adrenocorticotropin (ACTH) and α-melanocyte-stimulating hormone (α-MSH) in the rat brain. I. Origin of the extrahypothalamic fibers. Brain Res. 192: 433-440, 1980.

Negro-Vilar, A., and Saavedra, J.M.: Changes in brain somatostatin and vasopressin levels after stress in spontaneously hypertensive and Wistar-Kyoto rats. Brain Res. 5: 353-358, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00378-07 LCS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Electron Microscopic Study of the Uptake of the Antibody to Dopamine-B-Hydroxylase in the Rat Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	John Olschowka	Staff Fellow	LCS	NIMH
	Bang H. Hwang	Professor	Penn. State Univ.	

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	--

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Preparations are underway to study the retrograde flow of the antibody to dopamine-B-hydroxylase (ADBH) at the electron microscopic level. Following the intraventricular injection of the ADBH, noradrenergic cell bodies of the locus coeruleus will be studied for the localization of the DBH that had undergone retrograde flow from the terminal varicose areas of the rat brain. Following fixation, the sections will be stained by the peroxidase-antiperoxidase method.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00382-07 LCS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Localization and Characterization of Brain Neuropeptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
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	John Olschowka	Staff Fellow	LCS	NIMH
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	Margery C. Beinfeld	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.8	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Highly specific and sensitive radioimmunoassays coupled to HPLC and gel filtration techniques were utilized to identify secretin, motilin and bovine pancreatic peptide-like immunoreactivity in the brain of the rat. Immunocytochemical studies revealed the presence of motilin-like immunoreactive nerve fibers and cell bodies in the hypothalamus and preoptic areas. Pancreatic polypeptide (PP) immunoreactive cell bodies and nerve fibers were widely distributed in the rat brain. Large numbers of PP-immunoreactive cells were observed in the arcuate nucleus with scattered cells in the cortex, neostriatum, nucleus accumbens, nucleus interstitialis stria terminalis and nucleus preopticus medialis.

Project Description:

Objectives: 1) Identification of secretin in the rat and pig brain and determination of the role of this peptide in the central nervous system of the rat. 2) Identification, characterization and immunohistochemical localization and mapping of motilin-like immunoreactivity in the brain of the rat. Characterization of 4 antibodies to synthetic porcine motilin. 3) Identification of bovine pancreatic polypeptide (PP) immunoreactive cell bodies and nerve fibers in the brain.

Methods Employed: 1) Secretin and motilin radioimmunoassay. 2) High pressure liquid chromatography (HPLC). 3) Gel Filtration. 4) Microdissection of rat brain. 5) Immunocytochemistry of motilin and PP.

Major Findings:

(A) Secretin. 1) Identification of secretin-like immunoreactivity (SLI) in the brain of rat and pig. High pressure liquid chromatogram of tissue extracts showed that SLI of the rat brain coeluted precisely with SLI of rat duodenum, but slightly ahead of SLI of pig brain and duodenum and synthetic porcine secretin. The difference in elution pattern suggests that SLI in the rat is slightly different from SLI in the pig. 2) Gross dissection revealed a wide distribution of SLI in rat brain. The highest concentrations were observed in the pineal and pituitary glands, followed by the thalamus, hypothalamus and olfactory bulb. Lower concentrations were observed in the cerebellum, midbrain, septum striatum and hippocampus. The medulla-pons and the cerebral cortex contained the lowest concentrations.

(B) Motilin. 1) Identification and characterization of motilin in the rat brain and duodenum. Motilin-like immunoreactivity was identified and characterized in rat brain and duodenum by combining gel filtration and high pressure liquid chromatography (HPLC). Two major immunoreactive components were revealed - one with a retention time similar to, but not identical with, standard porcine motilin - the other, with a retention time consistent with a high molecular weight protein. This high molecular weight compound suggests the possible existence of a prohormone for motilin. The rat brain and duodenum showed identical HPLC retention times for the motilin immunoreactive components. 2) Characterization of 4 antisera to synthetic porcine motilin. The binding parameters of 4 rabbit antimotilin sera raised against synthetic porcine motilin were studied. All antisera exhibited a high degree of specificity for synthetic porcine motilin. Five synthetic motilin fragments were tested against the antisera. Two of the antisera reacted strongly with the N-terminal sequence and the other 2 antisera reacted with the C-terminal sequence of amino acids. The C-terminal directed antisera revealed immunoreactive motilin-like material in extracts of the rat forebrain, cerebellum and duodenum. One of the N-terminal directed antiserum detected 75% less motilin immunoreactivity while the other antiserum was unable to detect any immunoreactivity in tissue extracts. It would appear that the N-terminal region of rat motilin is different from the N-terminal of porcine motilin. 3) Histochemical localization and mapping of motilin-like immunoreactivity. With the use of a C-terminally directed antiserum, immunofluorescent varicose processes could be seen primarily within the hypothalamus, preoptic area and mammillary body. A rich innervation of varicose fibers was observed in the median eminence and organum vasculosum lamina terminalis (OVLT). Low densities of varicose fibers were observed along the basal hypothalamus,

preoptic area, periventricular area and amygdala. Moderate densities of varicosities were evident in the medial preoptic nucleus, nucleus interstitialis stria terminalis and nucleus dorsomedialis. A sparse number of cell bodies could be identified in the OVLT region of colchicine-treated rats. Following the use of an N-terminally directed antiserum many fluorescent cell bodies were revealed in the mediobasal hypothalamus. With this antiserum varicose fibers were generally observed in smaller quantity than that recorded for the C-terminally directed antiserum. However, much larger numbers of fibers were noted in the nucleus dorsomedialis, median forebrain bundle, paraventricular nucleus and ventral portion of the nucleus intersititialis stria terminalis.

(c) Bovine Pancreatic polypeptide. Distribution of PP immunoreactive neurons and nerve fibers in the central nervous system. In the forebrain PP immunoreactive cell bodies were observed in large numbers in the arcuate nucleus with scattered cells in the olfactory tubercle, cortex (layers II-VI), neostriatum, nucleus accumbens, nucleus preopticus medialis, nucleus interstitialis stria terminalis, and in the area of the medial forebrain bundle. Delicate, varicose, PP nerve fibers were widely distributed, but were very numberous in several subcortical areas -- nucleus accumbens, nucleus interstitialis stria terminalis, nucleus preopticus medialis, nucleus suprachismaticus, periventricular thalamic and hypothalamic nuclei, paraventricular nucleus and nucleus dorsomedialis. Caudally PP cells were found in regions which contain catecholamine cell groups -- the locus coeruleus (A6), the nucleus tractus solitarius (A2) and in the area of the nucleus retricularis (A1). Nerve fibers were especially dense in the nucleus parabrachialis dorsalis, nucleus tractus solitarius and the substantia gelatinosa trigemini.

Significance to Biomedical Research and the Program of the Institute: The basic neuroanatomical and immunochemical studies reported here lay the groundwork for a rational approach to studying the role of motilin, secretin and pancreatic polypeptide in central nervous system physiology.

Proposed Course of the Project: Motilin, secretin and PP concentrations will be determined in discrete nuclei of the rat and human brain. Studies on the diurnal rhythm of the 3 peptides will be undertaken. Further studies are in progress to determine the biochemical levels of PP within various regions of the brain.

Publications:

Charlton, C.G., O'Donohue, T.L., Miller, R.L. and Jacobowitz, D.M.: Secretin immunoreactivity in rat and pig brain. Peptides, 1981, in press.

O'Donohue, T.L., Charlton, S.G., Miller, R.L., Boden, G. and Jacobowitz, D.M.: Identification, characterization and distribution of secretin immunoreactivity in rat and pig brain. Proceedings of the National Academy of Science, 1981, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00388-05 LCS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Neurochemical and Histochemical Studies of Cholinergic Pathways of the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	Andrej Rotter	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	--

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The nucleus tegmentalis dorsalis lateralis (ntdl) is an integral part of the limbic midbrain area. We have previously presented evidence that this nucleus contains cholinergic cell bodies. Therefore, the cholinergic projection sites of the ntdl were examined in rats by a biochemical technique. The ntdl was destroyed unilaterally and brains were assayed after 14 days survival for changes in choline acetyltransferase activity in discrete brain areas. A bilateral projection appears to exist to the anteroventral and the posterior nucleus of the thalamus. An ipsilateral projection to the lateral portion of the medial thalamic nucleus and the ventral lateral geniculate body was found. The ntdl cholinergic projection appears to be a principal pathway that interrelates the thalamic area of the limbic forebrain with the dorsal tegmental area of the limbic midbrain region and therefore may play a functional role in emotional behavior.

Project Description:

Objectives: To investigate the possible cholinergic projection sites of innervation from the nucleus tegmentalis dorsalis (ntdl) to the forebrain of the rat.

Methods Employed: 1) Unilateral stereotaxic lesion of the ntdl. 2) Microdissection of brain nuclei. 3) Radioenzymatic assay for choline acetyltransferase (ChAT).

Major Findings: Following a unilateral stereotaxic lesion of the ntdl (14 days) significant decreases in ChAT activity were found bilaterally in the anteroventral and the posterior nucleus of the thalamus. An ipsilateral projection to the lateral portion of the medial thalamic nucleus and the ventral lateral geniculate body was found.

Significance to Biomedical Research and the Program of the Institute: The anteroventral thalamic nucleus is of interest because of its primary innervation of the cingulate gyrus ("limbic cortex") which is classically identified as part of Papez's circuit which was included in his theory of limbic function in emotional behavior. The ntdl may exert an important modulating influence on brain areas that influence emotional and autonomic behavior.

Proposed Course of the Project: Work is currently underway to investigate cholinergic projection sites of the tractus diagonalis of the rat brain.

Publications:

Rotter, A. and Jacobowitz, D.M.: Neurochemical identification of cholinergic forebrain projection sites of the nucleus tegmentalis dorsalis lateralis. Brain Res. Bull. (In Press), 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00394-04 LCS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Role of Central Cholinergic Mechanism in the Feedback of Gonadol Steroids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Historpharm. Sect.	LCS	NIMH
	Eric A. Muth	Biologist	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.1	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In a previous study, both the n. tractus diagonalis (td), a cholinergic cell body area, and the ventral tegmental area (avt), the location of the A10 dopaminergic cell bodies, showed changes in cholinergic activity in male and female rats after gonadal hormone manipulations which altered LH secretion. The neuropharmacological relationship of the td and the avt was explored in another series of experiments. Acute stimulation of the avt resulted in reduced acetylcholine concentration in the hippocampus, a well known td cholinergic projection area. In addition, both destruction of afferent catecholamine terminals in the td by 6-OHDA, and also chronic treatment with the dopamine receptor antagonist, haloperidol, caused alteration in choline acetyltransferase and acetylcholine concentration respectively, in the avt. These results suggest the presence of a feedback-type functional relationship between the td and the avt which may be pertinent to the cholinergic and dopaminergic neurotransmitter mediated effects of hormones and psychoactive drugs on behavior.

Project Description:

Objectives: To delineate the functional interaction between the mesolimbic dopamine system originating in the avt and the cholinergic system originating in the td.

Methods Employed: 1) Electrical stimulation of the avt. 2) Stereotaxic DA, 6-OHDA injection of the td. 3) Focused microwave irradiation of heads of rats used for Ach microassay. 4) Microdissection of brain nuclei. 5) Microenzymatic assays for ChaT, Ach, DA, NE. 6) Radioimmunoassay of LH in plasma.

Major Findings: 1) Neither stimulation nor ablation of the avt or the td had any effect on LH secretion. Acute stimulation of the avt resulted in reduced acetylcholine concentration in the hippocampus. 2) Both destruction of afferent catecholamine terminals in the td by 6-OHDA, and also chronic treatment with the dopamine receptor antagonist halopendol, caused alterations in ChAT and Ach concentration, respectively, in the avt.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the functional interaction of the td cholinergic system with the mesolimbic DA system may help to explain the effects of the neuroleptics and other psychotopic drugs known to exert their effects via dopaminergic and cholinergic systems.

Proposed Course of Project: Terminated.

Publications:

Muth, E.A., Crowley, W.R., Jacobowitz, D.M.: Effect of gonadal hormone manipulation on plasma luteinizing hormone and on choline acetyltransferase activity and acetylcholine levels in discrete nuclei of the rat brain. Neuroendocrinology 30: 329-336, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00396-03 LCS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies on Substance P as a Neurotransmitter in the Nucleus Tractus Solitarius

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	Cinda J. Helke	Guest Worker	LCS	NIMH
	Joseph A. DiMicco	Guest Worker	LCS	NIMH
	Nguyen B. Thoa	Pharmacologist	LCS	NIMH
	Irwin J. Kopin	Chief	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The influence of neonatally administered capsaicin on the substance P (SP) containing nerves within the nucleus tractus solitarius (NTS), the spinal trigeminal nucleus (NTV), spinal cord and various nuclei in the midbrain and forebrain was studied. Three months after injection the SP content of the dorsal horn of the spinal cord and the NTV was markedly decreased while the NTS and the midbrain and forebrain nuclei were unaffected.

Studies of the effect of capsaicin on efflux of SP from tissue slices of NTS, NTV and the hypothalamus revealed that capsaicin elicites release of SP from the NTS and NTV. No alteration of the efflux of SP from hypothalamic slices occurred following capsaicin. The differential sensitivity of neuronal regions to capsaicin suggests a morphological and/or neurochemical difference between sensory and nonsensory SP fibers.

Project Description:

Objectives: 1) To determine the pattern of depletion of SP in discrete nuclei of the brain and spinal cord of the rat following neonatally administered capsaicin. 2) To determine whether SP is released from NTS and NTV slices in vitro with potassium depolarization in a calcium dependent manner and to assess the ability of capsaicin to release SP from the NTS and NTV.

Methods Employed: 1) S.C. injection of capsaicin (50 mg/kg) into 2 day old rats. 2) Microdissection technique for removal of discrete nuclei. 3) Radioimmunoassay of SP. 4) Stimulation of the release of endogenous SP from rat brain slices in vitro.

Major Findings: 1) 3 months after the injection of neonatal rats with capsaicin, the SP content in the dorsal horn of the thoracic spinal cord was reduced by 43% while the SP content of the ventral horn was unaffected. Medullary nuclei also demonstrated a differential sensitivity to the SP depleting actions of neonatally administered capsaicin, the SP content of the spinal trigeminal nucleus and tract was decreased by 46% while the nucleus tractus solitarius was unaffected. No alterations in the levels of SP were detected in any of the midbrain and forebrain nuclei analyzed. 2) SP-like immunoreactivity is released from the NTV and the NTS in response to K+ depolarization and capsaicin (3.3×10^{-5} M). No alteration of the efflux of SP from hypothalamic slices occurred following capsaicin. However, the hypothalamic slices were responsive to K+.

Significance to Biomedical Research and the Program of the Institute: These are important findings in terms of revealing different populations of substance P neurons in the brain and spinal cord and elucidating the site of action of capsaicin, the active ingredient in various food products (paprika, chili peppers).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00397-03 LCS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Studies of α-Melanocyte Stimulating Hormone Multi-neurotransmitter Neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Histopharm. Sect.	LCS	NIMH
	Thomas L. O'Donohue	Staff Fellow	LCS	NIMH, NIGMS
	Gail E. Handelmann	Staff Fellow	LCS	NIMH
	Y. Peng Loh	Staff Scientist	LN	NICHD
	David S. Olton	Professor of Psychology	Johns Hopkins Univ.	

COOPERATING UNITS (if any)
Laboratory of Neurobiology, NICHD

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	0.7	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Previous studies demonstrated the existence of both a-MSH and deacetylated α-MSH in the same neurons in rat and human brain. The present studies demonstrate that α-MSH was 2-3 orders of magnitude more behaviorally potent than the deacetylated peptide in a behavioral paradigm. An enzyme which acetylates α-MSH was identified and the regional distribution was investigated. Highest enzyme activities were found in the pituitary gland and brain. Pituitary enzyme activities could be induced by physiological conditions which induce α-MSH synthesis. The role of the enzymatic acetylation appears to be in potentiating the α-MSH action by decreasing susceptability to peptidase degradation since α-MSH has a 5-10 fold longer half-life than deacetylated α-MSH in vitro. Another MSH related peptide γ-MSH has recently been identified in α-MSH-containing neurons in brain. The behavioral activity of the peptide was examined in a series of studies. Interestingly, γ-MSH had an opposite behavioral action from α-MSH in that α-MSH facilitated learning of visual discrimination tasks while γ-MSH markedly debilitated performance on the maze. The results of these studies demonstrate that one neuron can secrete multi-neurotransmitters with dramatically different behavioral actions. The secretory product of the multi-neurotransmitter neuron may be regulated by post-translational processing such as enzymatic acetylation.

Project Description:

Objectives: Our previous investigations have demonstrated that α-MSH is secreted from neurons in the brain which also contain, synthesize and secrete deacetylated α-MSH, β-endorphin, acetylated β-endorphin and γ-MSH. The objectives of these studies were 1) to examine the relative behavioral and physiological actions of the three MSH peptides; 2) determine the mechanism of N-acetylation of α-MSH; 3) determine the regional distribution of the acetylating mechanisms; 4) investigate whether N-acetylation can be physiologically induced.

Methods Enployed: 1) α-MSH radioimmunoassay; 2) High pressure liquid chromatography; 3) Intraventricular injection of MSH and observation of excessive grooming behavior; 4) Testing of attentional processes after MSH administration; 5) Anolis skin bioassay for melanotropic action of MSH; 6) Development of assay for α-MSH acetylating enzyme activity; 7) Dissection of rat to study distribution of acetylating enzyme.

Major Findings: 1) Relative behavioral and physiological potencies of α-MSH, deacetylated α-MSH and γ-MSH. Both α-MSH and deacetylated α-MSH were potent agonists in inducing Anolis melanocyte darkening in vitro, induced excessive grooming after intraventricular administration and facilitated performance on acquisition and reversal on a visual discrimination task after intraperitoneal administration. The acetylated form of α-MSH, however, was 2-3 orders of magnitude more potent behaviorally than deacetylated α-MSH. The difference in potency may primarily be due to the fact that N-acetylated α-MSH has a half-life 5-10 times longer than deacetylated α-MSH in brain homogenates in vitro. In contrast to α-MSH, γ-MSH was a very weak agonist in inducing Anolis melanocyte darkening in vitro, was ineffective in inducing excessive grooming and delayed reversal learning on the visual discrimination task. The results demonstrate that similar peptides secreted from the same neuron can have dramatically different and sometimes opposite behavioral actions. 2) Determination of the mechanism of α-MSH N-acetylation. Incubation of deacetylated α-MSH and acetyl[^{3}H]-Coenzyme A with an enzyme supernatant of rat brain produced [^{3}H]-acetyl-α-MSH. The enzymatic acetylation was heat sensitive and could be abolished by boiling homogenates containing acetylating enzyme activity. 3)Distribution of enzyme. The acetylating enzyme had a widespread distribution in the rat but highest activities were present in the brain and pituitary gland where the peptide was also selectively distributed. The widespread distribution, however, indicated that the enzyme was not solely localized to α-MSH synthesizing neurons and cells. 4) Physiological induction of enzyme activity. Although the α-MSH acetylating enzyme may not be located solely in α-MSH synthesizing cells, it may be important in α-MSH biosynthesis in cells that do synthesize the peptide. In order to determine if enzyme activity could be induced by manipulations which induce α-MSH biosynthesis, frogs were housed in chronic dark or light to either induce or suppress α-MSH biosynthesis. Housing in chronic dark induced both α-MSH acetylating enzyme activity and α-MSH biosynthesis compared to frogs housed in chronic light. It appears, therefore, that induction of α-MSH acetylating enzyme may be an important regulatory step in the biosynthesis of acetylated α-MSH.

Significance to Biomedical Research and the Program of the Institute:
The α-MSH containing neuron provides the first model of a multi-neuro-

transmitter neuron. It is hypothesized that many neurons may actually be multineurotransmitter neurons with the other co-transmitters yet to be discovered. The study of the pre- and post-synaptic regulation and action of the α-MSH-containing neuron should provide a first insight into the basic functioning of multi-neurotransmitter and multi-hormonal cells.

Proposed Course of Project: Further studies to understand pre- and post-synaptic regulation of the multi-neurotransmitter neuron are in progress.

Publications:

O'Donohue, T.L., Handelmann, G.E. , Loh, Y.P., Olton, D.S., Leibowitz, J. and Jacobowitz, D.M.: Comparison of Biological and Behavioral Activities of Alpha- and Gamma-Melanocyte Stimulating Hormones. Peptides 2: 101-104, 1981.

O'Donohue, T.L., Charlton, C.G., Thoa, N.B., Helke, C.J., Moody, T.W., Pert, A., Williams, A., Miller, R.L. and Jacobowitz, D.M.: Release of Alpha-Melanocyte Stimulating Hormone into Rat and Human Cerebrospinal Fluid in Vivo and from Rat Hypothalamus Slices in Vitro. Peptides 2, 93-100, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00401-16 LCS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Formation, Release, Disposition, and Metabolism of Neurotransmitters in Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief, LCS	LCS NIMH
OTHER:	David C. Jimerson	Medical Officer, Psychiatry	BP NIMH
	David Lozovsky	Visiting Scientist	LCS NIMH
	Virginia K. Weise	Chemist	LCS NIMH
	Isamu Yamaguchi	Guest Worker	LCS NIMH
	Giora Feuerstein	Visiting Associate	LCS NIMH
	Zofia Zukowska-Grojec	Visiting Fellow	LCS NIMH
	Mohamed Bayorh	Visiting Fellow	LCS NIMH

COOPERATING UNITS (if any)
Biological Psychiatry Branch, NIMH; Sections on Histopharmacology and Pharmacology, LCS, NIMH

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Medicine

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	3.8	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to elucidate the biochemical mechanisms controlling the synthesis, storage, release, action and termination of action of neurotransmitters and to determine how genetic factors, environmental stimuli or drugs influence these control mechanisms. This year particular attention has focused on the interactions of prostaglandins with mechanisms which maintain cardiovascular function after hemorrhagic or endotoxin-induced shock. The release of renin appears to be enhanced by prostaglandins and seems essential for cardiovascular recovery when sympathetic mechanisms fail. Conversion of arachidonic acid to various physiological active compounds (prostaglandins, thromboxone, and leukotrienes) has been examined in SHR and WKY rats; the differences in effects of arachidonic acid appear to be a result of differences in reactivity of the animals to the metabolic products rather than due to differences in arachidonate metabolism.

PROJECT DESCRIPTION:

Stimulation of the sympathetic outflow from the spinal cord of pithed rats affords a means of evaluating, in the absence of compensatory cardiovascular reflexes or drug effects on the central nervous system, the release of norepinephrine from the sympathetic nerves into an intact circulation and the pressor responses elicited by the released catecholamines. Previous studies had shown that spontaneously hypertensive (SHR) rats release normal quantities of catecholamines but have excessive pressor responses to sympathetic stimulation. As in SHR rats, sympathetic stimulation evokes excessive pressor responses in renal hypertensive rats but not in DOCA-salt treated rats. In both types of experimental hypertension the plasma catecholamine responses to sympathetic stimulation are normal. Excessive responses to administered norepinephrine confirms the conclusion that peripheral organ responsivity is a significant factor in the development and maintenance of hypertension in these animal models (Drs. DiMicco and Kopin). Picrotoxin (a GABA antagonist) evokes similar increases in plasma catecholamines in SHR and WKY rats, but SHR rats have a greater pressor response. Conversely, the reduction in blood pressure evoked by muscimol, a GABA agonist, is greater in SHR than WKY rats (Drs. Feuerstein, Yamaguchi, and Kopin).

Prostaglandin (PG) infusions influence various cardiovascular reflex responses (see below) but in pithed rats various PG's were not found to have significant effects on sympathetic stimulation-induced release of catecholamines from the adrenal medulla or the sympathetic nerves. Thus the effects which were found must be attributed to direct actions of the PG compounds on the arteriolar smooth muscle (PGE_2 and PGI_2) or on the heart (PGI_2) of the pithed rats (Drs. Feuerstein and Kopin).

Hemorrhage elicits reflex sympatho-adrenal medullary release of catecholamines which are increased in plasma of anesthetized or awake rats. Prostaglandins of the E series potentiate the catecholamine responses and accelerate recovery of blood pressure and heart rate. These effects appear to be mediated by a renal factor, presumably the renin-angiotensin system, since they are not seen in nephrectomized animals (Drs. Feuerstein and Kopin). When angiotensin formation is blocked by the administration of captopril, recovery of anesthetized rats from a moderate hemorrhage is completely prevented; plasma epinephrine and vasopressin levels rise to higher levels, but appear to be ineffective in returning the blood pressure to normal levels (Drs. Zerbe, Feuerstein, and Kopin). Prostaglandin I_2 (PGI_2) infusion facilitates the recovery from hemorrhage in anesthetized intact or adrenal-medullectomized-splanchnicectomized rats. When the adrenals are intact, PGI_2 enhances the catecholamine and vasopressin responses to hemorrhage; these appear sufficient since renin levels are not increased. When the adrenals are not intact, however, plasma epinephrine and vasopressin levels do not increase, but there is a marked increase in plasma renin (Drs. Feuerstein, Zerbe, Meyer, and Kopin). Other factors may also play a role in recovery from hemorrhage since in halothane-anesthetized, captopril-pretreated rats, naloxone facilitates recovery but does not enhance catecholamine responses. This may be due to reversal of the anesthetic-induced depression of cardiovascular function so that similar studies in awake rats are in progress (Drs. Feuerstein and Kopin).

Since exogenous prostaglandins appear to influence secretion of various hormonal pressor agents, the effects of administration of the prostaglandin precursor, arachidonic acid (AA), on the blood pressure were examined in spontaneously hypertensive (SHR) and related normotensive (WKY) and other Wistar rats. After administration of AA, plasma levels of thromboxan B_2, 6-keto $PGF_{1\alpha}$, and catecholamines increased similarly in normotensive and hypertensive rats, but there was a greater fall in blood pressure in the hypertensive animals (Drs. Feuerstein, Zukowska-Grojec, Kouz, Utsunomiya, and Kopin). Leukotriene D_2, another metabolite of AA, after a short, initial pressor effect, lowered the blood pressures of SHR but not WKY rats (Drs. Feuerstein, Zukowska-Grojec and Kopin). Since catecholamine levels are not depressed by any of the AA metabolites, it is likely that the hypotensive effects are mediated by a direct action on blood vessels rather than by diminution of sympathetic nerve activity.

Endotoxin evokes a prolonged decrease in systemic blood pressure of anesthetized cats which is attended by increases in plasma levels of epinephrine, norepinephrine, and the prostaglandin metabolite, 6-ketoprostaglandin $F_{1\alpha}$. Pretreatment with indomethacin, which blocks prostaglandin formation, significantly attenuates the hypotensive effect of endotoxin while also diminishing the increases in plasma catecholamines and the prostaglandin metabolite. These results suggest that prostaglandins are responsible for the hypotension which evokes reflex increases in catecholamine secretion (Drs. Feuerstein, DiMicco, Ramu, and Kopin).

Using tertiary-butyl dimethylsilyl derivative, methods have been developed to assay by gas chromatography-mass spectroscopy levels of MHPG and DHPG in small volumes of plasma. Pharmacological blockade of reuptake of norepinephrine (NE) into sympathetic nerves diminishes stimulation-induced increments in plasma DHPG and MHPG (Drs. Jimerson, Yamaguchi and Kopin). When prostaglandin enhances stress-induced increases in plasma NE, the metabolites are also increased (Drs. Jimerson, Feuerstein and Kopin). These results suggest that the metabolites are formed from released NE which is recaptured by the sympathetic nerves.

Conversion of MHPG to sulfate and glucuronide conjugates in rats is the major metabolic fate of MHPG, but a small amount of MHPG is converted to VMA (Drs. Lozovsky and Kopin), but in man, oxidation to VMA is a major metabolic route. Inhibition of sulfate conjugate formation by administration of dinitrophenol increases glucuronide formation with only slight effects of MHPG conversion to VMA. Even in Gunn rats, which have a genetic lack of glucuronide formation, inhibition of sulfate conjugation enhanced only slightly oxidation of MHPG to VMA. This indicates that the alcohol dehydrogenase responsible for oxidation of the glycol is not an effective enzyme in rats and preferential formation of conjugates of MHPG in rats is not a result of greater efficiency of conjugation.

PUBLICATIONS:

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Feuerstein, G., Jimerson, D.C. and Kopin, I.J.: Prostaglandins, catecholamines, and cardiovascular responses to hemorrhage. Am. J. Physiol. 240: (Regulatory Integrative Comp. Physiol. 9:) R166-R174, 1981.

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Feuerstein, G., DiMicco, J.A., Ramu, A. and Kopin, I.J.: Effect of indomethacin on blood pressure and plasma catecholamine response to acute endotoxemia. J. Phar. Pharmacol. In press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00402-09 LCS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

CNS Regulation of Autonomic and Endocrine Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Anthony Zavadil	Guest Worker	LCS NIMH
	Charles Saller	Guest Worker	LCS NIMH
	David Lozovsky	Visiting Scientist	LCS NIMH
	Giora Feuerstein	Visiting Associate	LCS NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to define the regions of brain, the neuronal pathways, and the neurotransmitter systems which control endocrine, metabolic and autonomic responses to internal stimuli (e.g., blood glucose, O_2-tension, hormonal state, body temperature, etc.) and to various types of stress (immobilization, foot-shock, hemorrhage, etc.) and the effects of drugs on these systems. This year the role of prostaglandins in control of autonomic function and the effects of glucose levels on dopaminergic neurones has been studied.

PROJECT DESCRIPTION:

Integration by the central nervous system of information from baro-and chemo-receptors and other sensors of the internal environment results in reflex modulation of respiratory, metabolic, and cardiovascular function. By use of surgical techniques for lesioning or microinjection of appropriate pharmacological agents, specific neuronal pathways may be interrupted or stimulated. Microdissection and microassay of putative neurotransmitters and their metabolites, determination of glucose utilization, and direct measurement of firing rates provides information about relative activity of specific neuronal systems.

Dopaminergic neurones in brain appear to be sensitive to changes in blood glucose levels, decreasing their firing rate and releasing less dopamine (as indicated by a decrease in dopamine metabolites) when blood glucose is elevated (Drs. Saller and Chiodo). Even relatively small amounts of glucose, comparable to the amounts consumed during a meal or released by the liver during stress, suppress both the firing of dopaminergic neurons in the brain and central dopamine release. In addition, glucose administration can modify behavioral responses to drugs and the attending biochemical effects on central dopaminergic neurons. Haloperidol administration normally accelerates markedly the rate of impulse flow along dopaminergic axons and activates tyrosine hydroxylase, thought to be the rate-limiting enzyme in catecholamine synthesis. Although glucose administration can either prevent or suppress the increased firing rate of dopamine-containing neurons normally ellicited by haloperidol, it does not prevent tyrosine hydroxylase activation. Glucose administration potentiates the behavioral responses evoked by haloperidol-induced blockade of dopamine receptors and blocks the dopamine-mediated behavioral activation evoked by amphetamine (Drs. Saller and Kopin).

In hyperglycemic diabetic rats, striatal dopamine receptors are increased, consistent with dopaminergic neurone hypoactivity (Drs. Lozovsky, Saller, and Kopin). This is prevented by treatment with insulin to restore euglycemia.

The C^{14}-2-deoxyglucose method has been used in an attempt to map the baroreceptor pathway in the brainstem of the pentobarbital-anesthetized cat (Drs. DiMicco, Sokoloff and Kopin). Preliminary experiments suggest that electrical stimulation of one carotid sinus nerve (which results in activation of the baroreceptor pathway as evidenced by a fall in arterial pressure and heart rate) produces an asymmetric pattern of enhanced glucose uptake in areas of the brain thought to be involved in cardiovascular regulation. Glucose uptake was greater in the ipsilateral nucleus of the tractus solitarius (NTS) at the level of the obex (first synapse of primary baroreceptor afferents in the central nervous system) and in the nucleus ambiguous (secondary projection of the NTS and site of origin of cardiac vagal preganglionic fibers) than in the corresponding structures on the side contralateral to nerve stimulation.

PGE_2 injected into the lateral cerebral ventricle or directly into the dorsomedial or posterior hypothalamic nuclei evokes pressor responses attended by increases in plasma norepinephrine but not epinephrine (Drs. Feuerstein, Adelberg, Kopin and Jacobowitz). Intracysternal injections of PGI_2 elicit only mild responses and only at doses which are sufficiently large to have systemic effects when injected intravenously; when injected into the dorsomedial nucleus, PGI_2 may have a selective effect on heart rate (Drs. Feuerstein, Adelberg, Jacobowitz and Kopin). $PGF_{2\alpha}$ injections in the paraventricular, dorsomedial, or posterior hypothalamic nuclei evoke marked increases in heart rate and blood pressure attended by increases in plasma catecholamines (Drs. Feuerstein, Adelberg, Kopin and Jacobowitz). Higher doses of $PGF_{2\alpha}$ injected into the cerebral ventricles also elicit increases in heart rate, blood pressure, respiration and rectal temperature. Vagotomy has little effect on the responses, but hexamethonium blocks the pressor and temperature responses but not the heart rate or respiratory responses. Removal of the kidneys abolishes the respiratory, pressor, and temperature responses in hexamethonium-treated rats indicating that renin-angiotensin might be involved (Drs. Feuerstein, Adelberg, Jacobowitz and Kopin).

PUBLICATIONS:

Weinstock, M., Zavadil, A.P., Muth, E.A., Crowley, W.R., O'Donohue, T.L., Jacobowitz, D.M. and Kopin, I.J.: Evidence that noradrenaline modulates the increase in striatal dopamine metabolism induced by muscarinic receptor stimulation. Eur. J. Pharmacol. 68: 427-435, 1980.

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Saller, C.F. and Chiodo, L.A.: Glucose suppresses basal firing and haloperidol-induced increases in the firing rate of central dopaminergic neurons. Science 210: 1269-1271, 1980.

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Feuerstein, G., Adelberg, S.A., Kopin, I.J. and Jacobowitz, D.M.: Cardiovascular and sympathetic responses to $PGF_{2\alpha}$ injection into hypothalamic nuclei. Eur. J. Pharmacol. In press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00403-08 LCS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Biochemical Indices of Autonomic Function in Humans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief, LCS	LCS NIMH
OTHER:	Virginia Weise	Chemist	LCS NIMH
	Young Kim	Anesthesiologist	CC NIH
	Ronald Polinsky	Staff Physician	LCS NIMH
	Giora Feuerstein	Visiting Associate	LCS NIMH

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LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Medicine

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine (DA), norepinephrine (NE), and epinephrine (EPI) and their metabolites and dopamine-β-hydroxylase (DBH) are present in human plasma and cerebrospinal fluid (CSF). Levels of these substances reflect the balance between their release and removal. The increases in levels of NE and EPI with standing, exertion, stress, drugs, administration, etc., are used to examine sympathetic neuronal responsivity. The responses and level of activity of the sympathetic nervous system in cardiovascular disorders (hypertension, hypotension) a variety of psychiatric disorders, and after treatment with drugs are being examined.

PROJECT DESCRIPTION:

Blood pressure is normally maintained within normal limits by a complex system of autonomic reflexes and hormonal balances which are orchestrated by the central nervous system. Plasma levels of the catecholamines, renin, vasopressin and cortisol reflect activity of the sympathoadrenal medullary, renal, neurohypophysial and pituitary - adreno cortical system, respectively. Plasma levels of the catecholamines reflect acute changes in sympathetic activity because they are metabolized so rapidly and levels must be maintained by continuous secretion. Methods to facilitate estimation of turnover rates of the norepinephrine and assessment of its uptake and storage have been developed using high pressure liquid chromatography and electrochemical detection (HPLC-ED). This method replaces the radioenzymatic method when isotopically labelled compounds are administered to the patients and has been proven to yield results comparable to the radioenzymatic methods when applied to assay of norepinephrine and dopamine in human plasma (Drs. Goldstein, Feuerstein, and Kopin).

Studies of plasma catecholamines have been carried out in patients with hypertension and with various diseases or drugs which interfere with autonomic function and are associated with orthostatic hypotension. The results of our studies of hypertension, as well as those in other laboratories have been reviewed (Drs. Kopin, Goldstein and Feuerstein). In our studies, as well as in several other reports, levels of NE in hypertensive patients are similar to those in normal subjects and among normal subjects NE levels in plasma tend to increase with age. This age relationship is not apparent in the hypertensive population, because among the hypertensive subjects there are a few young patients with high levels of plasma NE. In contrast to these negative studies, in other studies, hypertensive patients have been found to have higher levels of plasma NE than in normotensive subjects. In these positive studies the relationship of plasma NE levels to age was not found. Analyses of the results of both groups of studies shows that the difference between the two groups is a result of a sampling difference in the normal subjects. In positive studies (in which hypertensives were found to have high levels of plasma NE) levels of NE in control normotensive subjects were significantly lower and had smaller variances than in negative studies. Positive and negative studies agreed on the levels in hypertensive subjects. The differences in control values appears to have been due to use in positive studies of medical personnel. Random normotensive persons, not familiar with hospital procedures, have similarly relatively elevated catecholamine levels as do hypertensive subjects during conditions of blood sampling. Further studies on turnover rates of catecholamines in plasma are in progress with the collaboration of the NHLBI.

Studies of patients with autonomic dysfunction have been carried out in collaboration with the Section on Experimental Therapeutics. The responsivity of these patients to intravenous administration of pressor agents varies with the type of autonomic disorder responsible for the hypotension. Patients with multiple system atrophy (MSA), who have various central nervous disorders and relatively normal resting levels of plasma norepinephrine, have greater

responses to pressor agents primarily because they are unable to reflexly modulate the responses. This is reflected by a steep dose-response curve, but no change in threshold levels of pressor agent. Patients without evidence of central nervous system disease appear to suffer from destruction of their peripheral sympathetic nerves and have low resting levels of plasma norepinephrine. These patients also have steep dose-response curves for pressor substances, but the threshold dose for norepinephrine is reduced also, indicating that they are supersensitive to this pressor agent (Drs. Polinsky, Kopin, Ebert and Mrs. Weise). Patients with chronic autonomic dysfunction may also lack adrenal medullary responses to hypoglycemia and other autonomic reflexly mediated responses. Neuropharmacological assessment of autonomic dysfunction in patients with chronic autonomic failure has continued (Drs. Polinsky and Kopin). Examination of the blood pressure following a standard test meal given to orthostatic hypotension patients reveals a precipitous drop in blood pressure which occurs within minutes of the onset of food intake. This is not accompanied by a rise in either plasma norepinephrine or epinephrine concentrations. Control subjects showed neither a drop in blood pressure nor a rise in plasma catecholamines. A study of the hormonal responses which occur following ingestion of the standard meal and may be responsible for the hypotension is in progress.

Measurement of ligand binding to alpha receptors of red blood cells and platelets is thought to parallel changes which occur at the vascular alpha receptor. A study of alpha receptors done in collaboration with Dr. Marian Kafka, BP, reveals that both IOH and MSA patients have an increased number of alpha receptors but only IOH patients have a decreased amount of prostaglandin-stimulated cyclic AMP. This latter finding is also seen in hypertensive patients and schizophrenics.

A study of CSF levels of neuron-specific enolase (collaboration with Dr. Paul Marangos) and two-dimensional protein electrophoresis is in progress (Drs. Polinsky, Goldman, Merril, and Ebert).

Investigation of norepinephrine kinetics in patients with autonomic dysfunction is in progress (Drs. Polinsky and Kopin). This test was initially performed by infusing intravenously at a constant rate of tritiated l-norepinephrine and ^{14}C-d-norepinephrine since animal studies suggested that the dextro isomer is not stored in sympathetic nerves. Initial results show that both isomers are taken up and the tritiated l-norepinephrine is removed more rapidly from the plasma following a steady-state infusion. Studies now underway include ^{3}H-isoproterenol (which is not taken up by the nerves) to allow comparison of its clearance with the two isomers of norepinephrine. It is anticipated that the differences in clearance and secretion rates will help to further elucidate the abnormalities in catecholamine metabolism between the peripheral and central forms of orthostatic hypotension and in hypertension.

Therapeutic trials with a variety of pressor drugs have been initiated; these include midodrine (long acting alpha agonist) and dihydroergotamine (an ergot venoconstrictor). A trial of indomethacin yielded a beneficial

response in 11 of 12 patients. This resulted in an overall increase of 37 mmHg in the standing mean blood pressure. The plasma norepinephrine was lower than normal in only IOH patients. Indomethacin had no significant effects of plasma norepinephrine or renin responses to postural change but did decrease urinary levels of prostaglandin-E-like immunoreactivity.

A study of sleep EEG patterns in autonomic dysfunction has been initiated (with Dr. Wallace Mendelson) because of the reported occurrence of sleep apnea in Shy-Drager syndrome. Simultaneous measurement of plasma catecholamines, vasopressin, and renin is in progress since the afferent pathway is the same but the efferent path exists at different levels of the nervous system (Drs. Polinsky, Zerbe and Kopin).

PUBLICATIONS:

Kopin, I.J., McCarty, R. and Yamaguchi, I.: Plasma catecholamines in human and experimental hypertension. Clin. Exp. Hypertens. 2(366): 379-394, 1980.

Lake, C.R., Polinsky, R.J., Güllner, H.-G, Ebert, M.H., Ziegler, M.G., Bartter, F.C. and Kopin, I.J.: Dissociation between central and peripheral noradrenergic activity in essential hypertension. Clin. Sci. 59: 229s-233s, 1980.

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Kim, Y.D., Jones, M., Hanowell, S.T., Koch, J.P., Lees, D.E., Weise, V. and Kopin, I.J.: Changes in peripheral vascular and cardiac sympathetic activity before and after coronary artery bypass operations: Interrelationships with hemodynamic alterations. Am. Heart J. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00404-10 LCS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Immunological Localization of GAD and CSD in Neurones and other Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Enrico Mugnaini	Chief, Lab. of Neuromorphology	State Univ.Connecticut
	Milton Brightman	Head, Sect. Neurocytology	NINCDS/LNNS NIH
	Elaine Neale	Research Physiologist	NICHD/CH NIH

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Medicine

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Immunohistological methods are being used to define the distribution in the nervous system and other tissues of cells (neurones, glia, etc.) which contain specific enzymes associated with particular functions (e.g., production of specific neurotransmitters, hormones, etc.). The ontogeny of the specific proteins and their role in growth, differentiation and regenerative processes are being examined using these techniques.

PROJECT DESCRIPTION:

Histological demonstration of the presence in a neurone or its nerve endings of an enzyme required for the biosynthesis of a neurotransmitter is highly suggestive and generally acceptable evidence that the neurone secretes that neurotransmitter. Thus localization by immunohistochemical methods of glutamic acid decarboxylase (GAD) in a particular neurone indicates that α-aminobutyric acid (GABA) is the neurotransmitter released from that neurone. Glutamic acid decarboxylase (GAD) has been purified by two-dimensional electrophoresis after it was irreversibly inhibited with radioactivity labelled "Suicide Inhibitor." The labelled enzyme was used to stimulate formation in sheep of an antibody to the enzyme. After the antibody to glutamic acid decarboxylase (GAD) was raised and characterized, it was used to study the enzyme in brain. Using immunocytochemical techniques on the light - and electron-microscopic level, GAD-immunoreactivity was localized specifically in different brain areas, including the cerebellum, olfactory bulb, cerebral cortex, basal ganglia and substantia nigra in rat brain. In order to reveal GABAergic pathways, GAD-immunoreactivity was also investigated in specific brain areas after injection of neurotoxic agents and selective neurosurgery. Studies on the functional anatomy of the GABAergic system of the rat and characterization of GABAergic components in different tissue culture systems are in progress. The GAD activity is present in the same cultured cells which take up and store ^{3}H-GABA.

Glutamic acid decarboxylase was compared with the enzyme activity of cysteine sulfinic acid decarboxylase CSD), the proposed biosynthetic enzyme for hypotaurine and taurine. CSD activity was separated in two enzyme activities, termed CSD I and CSD II. CSD I was found to be extremely similar to liver CSD, but CSD II was indistinguishable by biochemical and immunological techniques from GAD.

In the pituitary, GAD immunoreactivity was found in the neural and intermediate lobes. This GAD disappeared with stalk section, but CSD I activity remained, demonstrating the difference in location as well as specificity of the enzyme activities.

A double staining method to demonstrate simultaneously the coincident localization of GAD and somotostatin has been developed and these two substances shown to be present in the same cells of the nucleus reticularis of the thalamus.

PUBLICATIONS:

Oertel, W.H., Schmechel, D.E., Daly, J.W., Tappaz, M.L. and Kopin, I.J.: Localization of glutamate decarboxylase on line-immunoelectrophoresis and two-dimensional electrophoresis by use of the radioactive suicide substrate [2-^{3}H]-γ-acetylenic GABA. Life Sci. 27: 2133-2141, 1980.

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Oertel, W.H., Tappaz, M.L., Kopin, I.J., Ransom, D.H. and Schmechel, D.E.: Production of an antiserum to rat brain glutamate (GAD)/cystein sulfinate (CSD) decarboxylase. Brain Res. Bull. 5(2): 713-719, 1980.

Oertel, W.H., Schmechel, D.E., Weise, V.K., Ransom, D.H., Tappaz, M.L., Krutzsch, H.C., Kopin, I.J.: Comparison of cysteine sulphinic acid decarboxylase (CSD) isoenzymes and glutamic acid decarboxylase (GAD) in rat: Is brain CSD I identical with liver CSD and brain CSD II with GAD? Neuroscience. In press.

Oertel, W.H., Schmechel, D.E., Tappaz, M.L. and Kopin, I.J.: Production of a specific antiserum to rat brain glutamic acid decarboxylase (GAD) by injection of an antigen-antibody complex. Neuroscience. In press.

Oertel, W.H., Schmechel, D.E., Brownstein, M.J., Ransom, D.H., and Kopin, I.J.: Decrease of GAD-immunoreactive nerve terminals in the substantia nigra after kainic acid lesion of the striatum. J. Histochem. Cytochem. In press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00405-02 LCS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Aminergic Receptor Function in Vivo

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Tichomir Torda	Visiting Fellow	LCS NIMH
	Isamu Yamaguchi	Guest Worker	LCS NIMH
	Zofia Zukowska-Grojec	Visiting Fellow	LCS NIMH
	Mohamed Bayorh	Visiting Fellow	LCS NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Medicine

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to assess changes in properties (number, affinity) of receptors in response to alterations in the environment, drug administration, stress, or hormones, and to develop methods for assessing receptor function in intact animals, in vivo. Stress or isoproterenol-induced reduction of adrenoceptors can be prevented by administration of quinacrine. The α-adrenoceptor mediated pressor responses to administered norepinephrine are of the α_2 type whereas different α_1- adrenoceptors (in the junction) respond to norepinephrine released from sympathetic nerves.

PROJECT DESCRIPTION:

In rats, forced immobilization or repeated injections of isoproterenol results in subsensitivity of β_1-adrenoceptors and α-adrenoceptors. This has been demonstrated by showing that the numbers of receptors are decreased (Drs. Torda, Yamaguchi, Hirata, Kopin and Axelrod). There is a parallel decrease in the cardiac acceleration and increase in blood pressure produced by administered catecholamines. These changes in receptor density and responsiveness to catecholamines can be prevented by administration of quinacrine, an anti-malarial agent which blocks phospholipase A_2. These studies indicate that the effects of quinacrine of phospholipid turnover alter the changes in receptor function. Treatment with indomethacin for one week reduces the number of cardiac β-adrenoceptors and the cardiac response to administered epinephrine (Drs. Feuerstein, Torda and Kopin).

Examination of the effects of different α-adrenoceptor blocking agents on the pressor responses to sympathetic stimulation or to administered norepinephrine in pithed rats and comparison with the effects of these drugs on stimulation-evoked increases in plasma catecholamines has shown that α_1-type adrenoceptors mediate the pressor response to norepinephrine released at sympathetic nerve endings whereas, α_2-type adrenoceptors are responsible for the pressor effect of administered norepinephrine as well as presynaptic feedback regulation of norepinephrine release. The implication of these observations is that α_1-adrenoceptors are located at the junction of the sympathetic nerves with the post-synaptic membrane whereas α_2-receptors are extrajunctional (Drs. Yamaguchi and Kopin).

These observations have been extended to include additional specific α_1 and α_2-adrenoceptor blocking agents (Drs. Zukowska-Grojec, Bayorh, and Kopin). The effects of α_1 and α_2 adrenoceptor blocking agents on the pressor response to sympathetic stimulation are additive, indicating that administered norepinephrine reaches both type receptors. Desmethylimipramine and cocaine, which block reuptake of norepinephrine into sympathetic nerves, do not greatly potentiate the pressor response to stimulation of the sympathetic outflow from the spinal cord of pithed rats, but do prolong and potentiate the effects of administered norepinephrine. These results suggest that uptake is not an important means of terminating the action of norepinephrine released at α_1 adrenoceptors at the arterioral smooth muscles, but is important at α_2-adrenoceptors (Drs. Zukowska-Grojec, Bayorh, and Kopin).

PUBLICATIONS:

Yamaguchi, I. and Kopin, I.J.: Differential inhibition of alpha-1 and alpha-2 adrenoceptor-mediated pressor responses in pithed rats. J. Pharmacol. Exp. Ther. 214(2): 275-281, 1980.

Feuerstein, G., Torda, T. and Kopin, I.J.: Effect of indomethacin on cardiac β-adrenergic receptors. Eur. J. Pharmacol. 67: 469-472, 1980.

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Torda, T., Yamaguchi, I., Hirata, F., Kopin, I.J. and Axelrod, J.: Quinacrine-blocked desensitization of adrenoceptors after immobilization stress or repeated injection of isoproterenol in rats. J. Pharmacol. Exp. Ther. 216(2): 334-338, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00351-07 LCS

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Ebert	Chief, Sec. on Exp. Therapeutics	LCS NIMH
OTHER:	R. S. Burns	Staff Physician	LCS NIMH
	I. J. Kopin	Chief, Lab. of Clinical Science	LCS NIMH
	R. L. Hawks	Chemist	NIDA
	E. Gordon	Head, Unit on Clinical Biochemistry	LCS NIMH

COOPERATING UNITS (if any)

Section on Medicine, LCS; Unit on Clinical Biochemistry, LCS

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Experimental Theraeputics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to develop techniques for studying central nervous system monoamine metabolism in man, and to carry out pharmacokinetic studies of psychoactive drugs. Studies of the kinetics and clearance of deuterated homovanillic acid (HVA) from the circulation continued in Rhesus monkeys and patients. Problems with the application of this technique to study CNS dopamine turnover in man were resolved by further study of the kinetics and volume of distribution of the metabolite, HVA, before and after probenecid administration. The mono-hydroxy metabolites of phencyclidine (PCP) were found to be psychoactive in primate studies, and may account for the mixed stimulant and depressant effects of the parent drug. The plasma half-lives of the mono-hydroxy metabolites are about two hours, and the hydroxy-cyclohexane metabolite partitions rapidly into brain, achieving levels three times greater than those in plasma.

Project Description:

Objectives: (1) To develop techniques for studying kinetic parameters of central nervous system monoamine metabolism in man. (2) To carry out pharmacokinetic studies of psychoactive drugs. (3) To utilize these techniques to study patients with a variety of psychiatric, neuropsychiatric, and psychosomatic disorders.

Methods Employed: (1) Biochemical methods: fluorometric, gas chromatographic, and mass spectrometric methods are used for assay of endogenous catecholamine metabolites in tissues and body fluids. Mass spectrometric methods are used for assay of deuterium-labelled catecholamine metabolites administered exogenously or synthesized endogenously. Gas chromatographic and radio-immunoassay techniques are used for assay of drugs in body fluids (probenecid and d-amphetamine). (2) Physiological methods: As the project progresses, an increasing number of metabolic experiments will be carried out in patients. However, as new procedures are being developed, appropriate experiments will be carried out in rat brain tissue and in primates using serial sampling of blood, cerebrospinal fluid, and urine as necessary. To facilitate these preclinical in vivo experiments on brain metabolism, a small primate metabolic laboratory has been developed with a capacity to maintain chronic collection of lateral ventricle or lumbar cerebrospinal fluid from chaired Rhesus monkeys for days or weeks at a time.

Major Findings: The development of a clinical procedure to estimate the brain production rate of homovanillic acid (HVA) has been continued (Drs. Burns, Ebert, Kopin). The relationship between probenecid dose (40 - 200 mg/Kg, I.V. infusion) and the differential inhibition of HVA transport at the chorid plexus and the kidney is being studied in the monkey by intraventricular injection of d_2-HVA and ^{3}H-HVA and determination by kinetic analysis of it's secretion rate from CSF to blood, and blood into urine. Clinical studies of previously untreated Parkinsonian patients (who have a 50-80% deficit in brain dopamine) are planned to investigate the relationship between the brain production rate of HVA, the plasma HVA level, and the total body HVA production rate. The relative value of measurements of the lumbar CSF concentration, the 24-hour urinary excretion rate, and the plasma concentration of HVA as an index of brain dopamine metabolism will be studied in this patient group.

The CSF kinetics of HVA has been studied in the monkey in an effort to provide a better interpretation of clinical results using lumbar CSF samples (Drs. Burns, Ebert, Kopin). d_2- or d_5-HVA together with ^{14}C-Methoxy-Inulin, a marker of bulk flow, are injected (in 100 µl) one space above an indwelling lumbar spinal catheter and the concentration of both species is determined in timed CSF samples withdrawn from the catheter (100 µl volume). The local elimination rate of deuterated-HVA has a mean half-life (T1/2) of 40 minutes compared to a disappearance T1/2 of 6 to 7 hours for ^{14}C-Inulin. The rapid clearance rate of HVA in the lumbar subarachnoid space may be related to the anatomy of the cauda equina and its associated blood vessels. Lumbar CSF HVA levels appear to be a function not only of the delivery rate

from above, but also the local clearance rate. d_2-HVA was also injected into the lateral ventricle via an indwelling cannula and its appearance in lumbar CSF, blood and urine monitored. d_2-HVA was detected in plasma within 5 minutes and reached peak concentrations at about 60 minutes after injection. Fifteen percent of the injected dose was present in the 90 minute fractional urine sample. Transport of HVA from CSF to blood by a high capacity, choroid plexus system coupled with secretion by the kidney leads to rapid removal of ventricular CSF HVA from the body. Plasma HVA levels may better reflect short term changes in striatal dopamine metabolism than previously appreciated.

The activity and kinetics of the monohydroxy metabolites of phencyclidine (PCP) have been studied in animals (Drs. Burns, Ebert, Hawks (NIDA)). The 4-hydroxy-cyclohexane derivative (OH-CYC), the major metabolite in man, was found to be active in primates, producing an excited state with motor restlessness, increased startle response, and purposeless movements in the monkey. The 4-hydroxy-piperidine derivative (OH-PIP), the minor metabolite in man, possesses PCP-like activity, but was found to be 5 to 10 times less potent than PCP in the monkey. A gas chromatography-mass spectrometry assay was developed in our laboratory for the measurement of OH-CYC and OH-PIP in body fluids and tissue samples. The plasma half-lives of OH-CYC and OH-PIP in the monkey are approximately 2 hours, not significantly shorter than the 3 hour half-life for PCP. Less than 4% of the administered dose of either metabolite is excreted unchanged in urine, indicating extensive conjugation or further metabolism is required to eliminate these species. The tissue distribution of OH-CYC was examined in the rat. OH-CYC rapidly distributes into brain tissue, achieving peak levels within 5 minutes. The compound selectively partitions into the brain, resulting in levels up to three times greater than those in plasma. The disappearance half-lives of OH-CYC in brain and adipose tissue were similar to the plasma half-life. The presence of OH-CYC in high concentrations in brain together with its relatively long half-life and CNS stimulant properties may explain the mixed stimulant and depressant effects of the parent drug, PCP. Studies of the kinetics and metabolism of PCP in man are now scheduled to begin. The effects of PCP in man include prolonged recovery, suggesting the existence of slow metabolizers, and behavioral toxicity in the form of an excited state.

Significance of Biomedical Research: Assessing the rate of amine metabolite formation in animals and in patients provides information on the rate of amine utilization. The development of more quantitative methods of determining central nervous system amine utilization in man is essential for testing hypotheses regarding the role of amines in psychiatric, medical, and neurological disorders, and assessing the effects of drugs on amines in the brain. Studies of the kinetics, metabolism, and behavioral effects of PCP bear directly on the psychiatric and neurological consequences of abuse of this drug.

Proposed Course: The metabolic techniques described above for the study of catecholamine metabolism are now being applied to studies in normal volunteers and patient groups.

Pharmacokinetic studies of PCP and its mono-hydroxy and di-hydroxy metabolites in normal volunteers will be concluded. The behavioral pharmacology of the mono-hydroxy and di-hydroxy metabolites will be studied in animals. Studies of the tissue distribution of PCP metabolites will be continued.

Publications:

1. Burns, R.S. and Lerner, S.E.: The effects of phencyclidine in man: A review. In, Phencyclidine: Historical and Current Perspectives, Domino, E.F. (Ed.) NPP Books, Ann Arbor, Michigan, 1980.

2. Burns, R.S., Lerner, S.E., and Linder, R.L.: The clinical picture of phencyclidine intoxication. In, Current Topics II in Emergency Medicine, The Medical College of Pennsylvania Press, Philadelphia, Pennsylvania, 1980.

3. Mikkelsen, E., Lake, C.R., Brown, G.L., Ziegler, M.G., Ebert, M.H.: The hyperactive child syndrome: Peripheral sympathetic nervous system function and the effect of d-amphetamine. Psychiatry Research 4:157-169, 1981.

4. Lerner, P., Major, L.F., Ziegler, M., Dendel, P.S., Ebert, M.H.: Central noradrenergic adoption to long term treatment with imipramine in Rhesus monkeys. Brain Research 200(1):220-224, 1980.

5. Brown, G.L., Ebert, M.H., Hunt R.D., Rapoport, J.L.: Urinary 3-methoxy-4-hydroxyphenylglycol and homovannilic acid response to d-amphetamine in hyperactive children. Biological Psychiatry 16:779-787, 1981.

6. Ng, L.K.Y., Thoa, N.B., Ebert, M.H.: Behavioral and neurochemical effects of electroconvulsive shock in the withdrawal syndrome of morphine dependent rats. Acupuncture and Electro-Therapeutic Research 6:33-46, 1981.

7. Elchisak, M.A., Polinsky, R.J., Ebert, M.H., Kopin, I.J.: Kinetics of homovanillic acid and determination of its production rate in humans. Submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00352-06 LCS

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pharmacological and Psychometric Studies of Neuropsychiatric Syndromes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	R. J. Polinsky	Staff Physician	LCS NIMH
	W. H. Kaye	Staff Psychiatrist	LCS NIMH
	P. R. Martin	Visiting Scientist	NIAAA
	D. Goldman	Clinical Associate	LCS NIMH
	C. R. Merril	Sr. Research Scientist	LGCB NIMH
	H. Weingartner	Research Psychologist	LPP NIMH
	I. J. Kopin	Chief, Lab. of Clinical Science	LCS NIMH
	L. E. Nee	Clinical Social Worker	LCS NIMH

COOPERATING UNITS (if any)

Section on Medicine, LCS; Laboratory of Psychology and Psychopathology, NIMH; Laboratory of General and Comparative Biochemistry, NIMH; NIAAA

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Experimental Therapeutics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

XX (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several neuropsychiatric syndromes including Alzheimer's dementia, Gilles de la Tourette's syndrome, are being studied in terms of memory function and possible pharmacological treatment. The pathophysiology and neuropharmacology of anorexia nervosa and orthostatic hypotension is also under investigation. A clinical trial of a combination of lecithin and THA (an oral cholinesterase inhibitor) in Alzheimer's dementia was not efficacious. A single dose trial of DDAVP, a vasopressin analog, in Alzheimer's dementia increases semantic memory and arousal. A study of brain dopamine and serotonin metabolism in Gilles de la Tourette's syndrome did not indicate an abnormality in these systems. Endogenous opioids are increased in CSF in underweight patients with anorexia nervosa. In orthostatic hypotension therapeutic trials with midodrine (a long-acting alpha agonist) and dihydroergotamine (an ergot veno-constricter) are underway. A trial of indomethacin in orthostatic hypotension was efficacious, and raised standing mean blood pressure an average of 37 mm Hg.

Project Description:

Objectives: The purpose of this study is to investigate several neuropsychiatric syndromes from the perspective of memory and intellectual function, neurotransmitter metabolism, neuroendocrine function, and possible pharmacological treatment. Currently under study are several dementias with known neuropathology, particularly Alzheimer's dementia and Korsakoff's dementia. Other diseases under intensive study are orthostatic hypotension, anorexia nervosa, and Gilles de la Tourette's syndrome.

Methods Employed: Methods for studying information processing in neuropsychiatric disorders include learning tasks that permit the assessment of stimulus and modality specific information in processing (1) short-term memory; (2) long-term memory; (3) retrieval processing; (4) encoding; (5) the interrelationships of these proecesses; and (6) factors that might influence decay and interference of memory in these systems. Procedures are used to test free recall, serial learning, cued recall, and recognition memory.

One strategy for identifying neurotransmitter systems that may play a role in the pathogenesis of psychiatric or neurological symptoms is to study the clinical effects of a series of drugs having relatively specific stimulating or blocking effects on a particular neurotransmitter system. This approach has been profitably applied to research on affective disorders. We are pursuing a pilot investigation of this nature in patients with dementias of varying etiologies, focusing on the adrenergic and cholinergic system.

Methods used in neuroendocrine protocols are established techniques of studying plasma prolactin and growth hormone responses to the acute administration of drugs known to affect dopaminergic neurotransmission. From these endocrine responses, one can infer the functional state of dopaminergic transmission in the tuberoinfundibular systems. Methods used in Stage II drug trials are established methods for the evaluation of psychotropic drugs including double-blind design, appropriate rating scales, placebo controls.

Methods used in studies of neurotransmitter metabolism are those methods oulined in project ZO1 MH 000351-06 LCS.

Methods used to study protein abnormalities or search for genetic markers in psychiatric and neurological diseases involves the application of two-dimensional polyacrylamide gel electrophoresis of proteins. These two dimensional gels can be studied and quantitatively analyzed either by silver staining of the proteins present or by labeling the proteins in biological sample and performing radioautograms. Image processing computer systems are used to identify and quantitate the individual proteins appearing on the gel.

Major Findings: Neuropharmacological assessment of autonomic dysfunction has continued in patients with chronic autonomic failure (Drs. Polinsky & Kopin). Examination of the blood pressure following a standard

test meal given to orthostatic hypotension patients reveals a precipitous drop in blood pressure which occurs within minutes of the onset of food intake. This is not accompanied by a rise in either plasma norepinephrine or epinephrine concentrations. Control subjects showed neither a drop in blood pressure nor a rise in plasma catecholamines. A study of the hormonal responses which occur following ingestion of the standard meal is in progress.

Measurement of ligand binding to alpha receptors of red blood cells and platelets is thought to parallel changes which occur at the vascular alpha receptor. A study of alpha receptors done in collaboration with Dr. Marian Kafka, BPB, reveals that both IOH and MSA patients have an increased number of alpha receptors but only IOH patients have a decreased amount of prostaglandin-stimulated cyclic AMP. This latter finding is also seen in hypertensive patients and schizophrenics.

A study of CSF levels of neuron-specific enolase (collaboration with Dr. Paul Marangos) and two-dimensional protein electrophoresis is in progress in patients with autonomic dysfunction (Drs. Polinsky, Goldman, Merril, Ebert).

Investigation of norepinephrine kinetics in patients with autonomic dysfunction is in progress (Drs. Polinsky & Kopin). This test was initially performed by administering a constant-rate infusion of tritiated l-norepinephrine and ^{14}C-d-norepinephrine since animal studies suggested that the dextro isomer was not taken up by sympathetic nerves. Initial results show that both isomers are taken up and the tritiated l-norepinephrine is removed more rapidly from the plasma following a steady state infusion. Studies are now underway which include ^{3}H-isoproterenol which is not taken up by the nerves and will allow comparison of its clearance with the two isomers of norepinephrine. It is anticipated that the clearance and secretion rate will help to further elucidate the differences in catecholamine metabolism between the peripheral and central forms of orthostatic hypotension.

Therapeutic trials with a variety of pressor drugs have been initiated; these include midodrine (long acting alpha agonist) and dihydroergotamine (an ergot venoconstrictor). A trial of indomethacin yielded a beneficial response in 11 of 12 patients. This resulted in an overall increase of 37 mmHg in the standing mean blood pressure. The plasma norepinephrine was lower than normal in only IOH patients. Indomethacin had no significant effects of plasma norepinephrine or renin response to postural change but did decrease urinary levels of prostaglandin-E-like immunoreactivity.

A study of sleep EEG patterns in autonomic dysfunction has been initiated (with Dr. Wallace Mendelson) because of the reported occurrence of sleep apnea in Shy-Drager syndrome. Simultaneous measurement of plasma catecholamines, vasopressin, and renin is in progress since the afferent pathway is the same but the efferent path exists at different levels of the nervous system (Drs. Polinsky, Zerbe, Kopin).

In a collaborative effort with Dr. Merril (LGCB, NIMH), quantitative analysis of hundreds of proteins in CSF, lymphocytes, and other tissues and fluids is being developed to screen for genetic markers in psychiatric and neurological diseases. Two-dimensional electrophoresis separates the proteins, and either silver staining or autoradiography can be used to visualize hundreds of protein species in the resulting patterns. Patterns are digitalized with a high resolution densitometer and then measurement and analysis of charge, molecular weight, and relative concentration for each of the proteins detected in a sample is done with the assistance of a computer (Drs. Goldman, Merril, Ebert).

More than 300 protein sepcies were detected in 60 µl of human CSF. A "map" of the normal human pattern was established, with 26 proteins identified and 20 clusters of differences between plasma and CSF protein patterns indicated. In primates, large qualitative differences were found between ventricular, cisternal and lumbar CSF proteins.

The utility of the two-dimensional electrophoretic technique in searching for protein markers in genetic diseases was assessed by evaluating more than 400 proteins of ^{14}C-labelled lymphocytes from each of a small group of Lesch-Nyhan patients and controls. Eleven quantitative protein differences were found that distinguished patients from controls. Immunologic and enzymatic studies of hypoxanthine-guanine phosphoribosyltransferase, the enzyme which is defective in the Lesch-Nyhan disease, established that while antigen capable of cross-reacting with antibody made to normal HPRT was absent, there was still a nearly normal amount of HPRT protein present in two-dimensional gels and trace amounts of HPRT activity in erythrocyte lysates of the three patients studied.

A larger clinical study of protein markers in Huntington's Disease is nearing completion. Lymphocytes, erythrocytes, plasma, and CSF obtained from 30 individuals studied clinically on our ward have been obtained and processed by two-dimensional electrophoresis. Protein variations in Huntington's Disease are being catalogued against the background range of normal variations in protein charge, molecular weight, and density (Drs. Goldman, Polinsky, Ebert, Merril).

Neuropharmacological studies of Alzheimer's dementia continue, and a study of Korsakoff's psychosis has been initiated. Detailed neuropsychological studies of Alzheimer's dementia reveal that, although many intellectual functions are preserved (in the early stages of the disease), the patients are unable to access structures in memory that are necessary for organizing and effectively encoding ongoing events. They therefore fail to from retrievable trace events in long-term memory. Inaccessability of information in semantic memory can disrupt memory for episodic events. A chronic treatment protocol in which lecithin and THA (an oral cholinesterase inhibitor) were administered concurrently to 4 patients for up to one month did not result in long term improvement in memory function. An acute trial of DDAVP, a vasopressin analogue administered intranasaly, has been completed in 8 patients. Patients demonstrated a significant increase

in recall of words associated to a letter shortly after DDAVP administration along with concurrent changes in mood and motor activity that suggested increased arousal (Drs. Kaye, Weingartner, Gillin, Ebert).

A study of the relationship between central nervous system metabolism of biogenic amines and neuropeptides and neuropsychological function in Korsakoff's psychosis has been initiated (Drs. Martin, Ebert, Weingartner). In particular, the hypothesis that there is a central noradrenergic deficit in Korsakoff's will be studied. A therapeutic trial of clonidine (an α-2-noradrenergic agonist) has begun, and a trial of DGAVP (a vasopressin analog) is planned.

In Gilles de la Tourette's syndrome, a study of CSF amine metabolites was conducted to study brain dopamine and serotonin metabolism (Drs. Polinsky & Ebert). Preliminary data analysis does not reveal an abnormality in CSF HVA and 5HIAA compared with age and sex matched control subjects. A study of sleep physiology in Tourette's syndrome has been completed, and data analysis in progress (Drs. Mendelson, Gillin, Ebert, Polinsky). This study follows a preliminary finding by our group of a 30 per cent reduction of delta stage sleep in a small number of Tourette patients which returned to normal during haloperidol treatment.

A clinical and family study of 30 adult Tourette's syndrome patients revealed that 80 per cent of patients had a positive family history of Tourette's syndrome or simple tics. A high incidence of obsessive-compulsive behavior, sleep disturbance, and depression was noted. Forty per cent of patients experienced a peak in tics in the first decade after onset of symptoms (Ms. Nee, Drs. Polinsky, Ebert).

The clinical research program in neuropharmacological and neurochemical aspects of anorexia nervosa has continued this year. Naturalistic studies of eating behavior and physiological parameters have begun in patients with anorexia nervosa who are "free-feeding" on the research ward. Patients who are more than 25 per cent below ideal body weight and who are eating less than 1200 calories per day maintain levels of most essential plasma amino acids in the normal range. Similarly underweight patients with anorexia nervosa maintain a normal circadian rhythm of core body temperature, although the mean body temperature is subnormal (Drs. Kaye and Ebert).

The induction of REM sleep by arecholine has been utilized as a measure of central cholinergic responsivity in man. Compared to normal controls, patients with primary affective disorder during both the depressed as well as the euthymic state have a faster REM-induction response to arecholine challenge. A study of the cholinergic REM-induction response in patients with anorexia nervosa demonstrated that those patients with co-existing affective illness were significantly more sensitive to arecholine than those who did not have evidence of an affective disorder. We are investigating whether or not these patients have a different clinical course or response to pharmacological or behavioral treatment (Drs. Sitaram, Kaye, Gillin, Ebert).

Recent animal studies indicate that most endogenous opioids may play a role in the regulation of feeding behavior. A study of endogenous opioids in CSF (measured by radioreceptor assay) was carried out in patients in various stages of anorexia nervosa. CSF opioid activity was significantly elevated in patients at minimum weight (6.08 $\pm$ .9 pmol/ml) compared to the same patients after weight restoration (2.04 $\pm$ .9 pmol/ml). Opioid levels in normal controls and patients with chronic anorexia nervosa who were of normal weight were not different than the levels in acute patients after weight restoration (Drs. Pickar, Naber, Kaye, Ebert).

Significance of Biomedical Research: In recent years interest has increased in the biochemical aspects of learning and memory. Neuropharmacological aspects of memory function have also become a focus of interest. Most of the published studies in this area are in animals. We hope to extend knowledge about pharmacological and biochemical aspects of memory function by studying several diseases in which disordered memory is a primary symptom, and in which there is existing knowledge about anatomical and biochemical pathology. Gilles de la Tourette's syndrome is a neuropsychiatric syndrome whose pathophysiology is poorly understood. The symptomatology appears to be responsive to drugs which block dopamine receptors. By conducting new drug trials, and generating data on cognitive and linguistic function, monoamine metabolism, and neurological endocrine function in this illness, we hope to expand knowledge about it's pathophysiology. Anorexia nervosa is another neuropsychiatric syndrome in which there is little published information concerning pharmacological treatment and the neurotransmitter metabolism. It is clearly established in the animal literature that monoamine systems in the hypothalamus play an important role in the normal regulation of appetite. Orthostatic hypotension is a clinical syndrome that can now be divided into subtypes that have etiological and therapeutic implication using new techniques for studying catecholamine metabolism and physiology.

Proposed Course: In Alzheimer's dementia, a series of clinical trials of vasopressin analogs will take place. In Korsakoff's dementia, assessment of CNS norepinephrine metabolism and a clinical trial of clonidine is underway. In Gilles de la Tourette's syndrome, genetic studies will continue and further studies of dopamine metabolism will be carried out. In anorexia nervosa, neurochemical studies of CNS opiate, noradrenergic, and vasopressin systems will continue to evaluate their role in the pathophysiology of the syndrome. In orthostatic hypotension, clinical trials of midodrine and dihydroergotamine will continue. Studies of the kinetics and disposition of infused radio-labelled d- and l-norepinephrine will be carried out.

Publications:

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2. Polinsky, R.J., Kopin, I.J., Ebert, M.H., and Weise, V.: The adrenal medullary response to hypoglycemia in patients with orthostatic hypotension. Journal of Clinical Endocrinology and Metabolism 51:1401-1406, 1980.

3. Polinsky, R.J., Kopin, I.J., Ebert, M.H., Weise, V., and Recant, L.: Hormonal responses to hypoglycemia in orthostatic hypotension patients with adrenergic insufficiency. Life Sciences 29:417-425, 1981.

4. Tetsuo, M., Polinsky, R.J., Markey, S.P., and Kopin, I.J.: Urinary 6-hydroxymelatonin excretion in patients with orthostatic hypotension. Journal of Clinical Endocrinology and Metabolism, (in press).

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15. Weingartner, H., Kaye, W., Smallberg, S.A., Ebert, M.H., Gillin, J.C., Sitaram N.: Memory failures in progressive idiopathic dementia. Journal of Abnormal Psychology, (in press).

16. Kaye, W.H., Sitaram, N., Weingartner, H., Ebert, M.H., Smallberg, S., Gillin, J.C.: Modest facilitation of memory in dementia with combined lecithin and anticholinesterase treatment. Biological Psychiatry, (in press).

17. Kaye, W.H., Weingartner, H., Gold, P., Ebert, M.H., Gillin, J.C., Sitaram, N., Smallberg, S.: Cognitive effects of cholinergic and vasopressin agents in patients with primary degenerative dementia. In, Memory and Aging, Wurtman, R.J. (Ed.), Raven Press, New York, (in press).

18. Merril, C.R., Goldman, D., Ebert, M.: Protein variations associated with the Lesch-Nyhan syndrome. Proceedings of the National Academy of Science, (in press).

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20. Rapoport, J.L., Nee, L., Mitchell, S., Polinsky, R.J., Ebert, M.: Hyperkinetic syndrome and Tourette's syndrome. In, Proceedings of the First International Tourette Syndrome Symposium, Raven Press, New York, (in press).

21. Nee, L.E., Polinsky, R.J. Ebert, M.H.: Gilles de la Tourette's syndrome: A clinical and family study of 30 adults. In, Proceedings of the First International Tourette Syndrome Symposium, Raven Press, New York, (in press).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00424-06 LCS

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biological Active Peptides in the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI's:	M.Brownstein, D.K.Meyer, R.Pruss	LCS NIMH
	R.Eskay, L.E.Eiden	LCS NIMH
	M.C.Beinfeld, M.Palkovits	LCS NIMH
Other:	H.Gainer, J.Russell, Y.P.Loh	LDN NICHHD
	J.Roth, S.Taylor, R.Kahn, R.Jensen	IRP NIAMDD
	E.Schiffman	LDBA NIDR
	C.Bennett	Merck Co.
	J.Kebabian	NINCDS
	I.Phillips	U.Florida
	W.Vale	Salk Institute
	A.Arimura	Tulane U.
	P.Giraud	U.Marseilles, France
	E.A.Zimmerman	Columbia U.
	D.T.Krieger	Mt.Sinai

COOPERATING UNITS (if any)
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5.75		0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have continued to study the distribution of peptide-containing cells in the central nervous system, the biosynthesis of biologically active peptides, and the factors that regulate peptide secretion. Our studies of a number of peptides have contributed to a better understanding of the cell biology of peptidergic neurons and of their role in the brain.

PROJECT DESCRIPTION:

Objectives: To locate specific groups of neurons in the central and peripheral nervous systems which synthesize and secrete peptides; to determine the biochemical mechanisms underlying the synthesis of peptides by neurosecretory cells; to study interactions between peptidergic and nonpeptidergic neurons.

Methods Employed: Biosynthetic, enzymatic and histologic procedures; microdissection techniques; immunocytochemistry.

Major Findings: The distribution of insulin receptors in rat brain has been examined by a new autoradiographic method (Drs. Young, Kuhar, Roth, Brownstein). The density of receptors is especially high in fetal rats suggesting a role of insulin or a related molecule in CNS growth or development.

The details of regulation of -MSH secretion from the intermediate lobe of the pituitary by catecholamines has been described (Drs. Eskay and Kebabian). The structures of the -MSH-like peptides in brain have been investigated. Desacetyl -MSH has been demonstrated in brain extracts (Drs. Loh and Eskay).

A number of neuropeptides have been found in mammalian and amphibian retinae and, more recently, immunocytochemistry has been used to localize these peptides to specific cells (Dr. Eskay). Along similar lines, methods for dissociating and segregating retinal neurons have been developed (Drs. Schaeffer and Brownstein), and now large numbers of photoreceptor and ganglion cells can be prepared for biochemical studies.

The distribution of cholecystokinin (CCK) in the brain has been described and the immunologically active material in brain extracts characterized by high performance liquid chromatography (Drs. Beinfeld and Meyer). The studies have focused on the hypothalamo-neurohypophysial system and have shown that CCK is present in the posterior pituitary and released in parallel with oxytocin and vasopressin. A specific, high affinity binding site for CCK has been demonstrated to be present on brain membranes (Drs. Hays, Paul, Jensen, Beinfeld), and studies of distribution and regulation of receptors are under way.

The innervation of the supraoptic nucleus has been examined (Dr. Meyer) and a natriuretic factor partially

characterized. In a related study, the angiotensin-like material in gut and brain is being characterized (Drs. Furness, Phillips, Meyer).

Partial characterization of the LHRH-like peptide in frog sympathetic ganglion has been accomplished (Dr. Eiden). This peptide has received a great deal of attention from electrophysiologists; it is not identical to LHRH. Dr. Eiden is currently engaged in studying the regulation of enkephalin biosynthesis and release in cultured adrenal chromaffin cells.

The retinal toxicity of methylazoxymethanol (MAM), which has been found to produce a fairly unique retinal lesion in fetal and neonatal rats, is being studied by Drs. Eiden, Latker, and Zatz.

In a series of immunocytochemical studies in the gut, several peptides (e.g., somatostatin, bombesin) have been localized to specific neuronal groups. The immunoreactive species have been characterized by high performance liquid chromatography (Dr. Furness).

Significance to Biomedical Research: Peptides secreted by neurons in the CNS appear to act not only as factors which control the anterior pituitary but also as agents (neurotransmitters) which allow nerve cells to interact with one another. Thus it is important to develop methods for studying these compounds.

Proposed Course of Project: We hope to learn more about the growth and development of peptide containing neurons in the CNS, about the cell biology of these neurons, and about their interactions with other cell populations.

PUBICATIONS:

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Young, W.S., Kuhar, M.J., Roth, J., and Brownstein, M.J.: Radiohistochemical localization of insulin receptors in the adult and devleoping rat brain. Neuropeptides 1: 15-22, 1980.

Hays, S.E., Beinfeld, M.C., Jensen, R.T., Goodwin, F.K., and Paul, S.M.: Demonstration of a putative receptor site for cholecystokinin in rat brain. Neuropeptides 1: 53-62, 1980.

Skolnick, P., Paul, S., Zatz, M., and Eskay, R.: "Brain specific" benzodiazephine receptors and localized in the inner plexiform layer of rat retina. Eur. J. Pharmacol. 66: 133-136, 1980.

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Cote, T., Munemura, M., Eskay, R. and Kebabian, J.: Biochemical identification of the β-adrenoceptor and evidence for the involvement of a cyclic AMP system in the β-adrenergic-induced release of α-melanocyte stimulating hormone in the intermediate lobe of the rat pituitary gland. Endocrinology 107: 108-116.

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Beinfeld, M.C., Meyer, D.K., and Brownstein, M.J.: Cholecystokinin octapeptide in the rat hypothalamo-neurohypophysial system. Nature 288: 376-377, 1980.

Eskay, R.L., Furness, J.B., and Long, R.T.: Substance P activity in the bullfrog retina: localization and identification in several vertebrate species. Science (in press), 1981.

Beinfeld, M.C.: An HPLC and RIA analysis of the cholecystokinin peptides in rat brain. Neuropeptides 1: 203-209, 1981.

Meyer, D.K. and Helke, C.: Effect of chronic saline-treatment on cholinergic neurons in supraoptic and paraventricular nucleus. Neuropeptides (in press), 1981.

Giraud, P., Eiden, L.E., Audigier, Y., Gillioz, P., Conte-Devolx, B., Boudouresque, F., Eskay, R., and Oliver, C.: Enkephalins, ACTH, α-MSH and β-endorphin in human pheochromocytomas. Neuropeptides 1: 237-252, 1981.

Brownstein, M.J., Russell, J.T., and Gainer, H.: Biosynthesis of posterior pituitary hormones. In L. Martini and W. Ganong (Eds.): Frontiers in Neuroendocrinology. New York, Raven, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00271-12 LCS

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Measurement of Brain Contribution to Catecholamine Metabolites in Human Urine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief, LCS	LCS NIMH
OTHER:	Edna K. Gordon	Chemist	LCS NIMH
	Michael H. Ebert	Chief, Section on Expmtl. Ther.	LCS NIMH
	Jerry A. Oliver	Chemist	LCS NIMH
	Robert L. Sherman	Chemist	LCS NIMH
	Ib Oddershede	Visiting Fellow	LCS NIMH
	Sanford P. Markey	Pharmacologist	LCS NIMH

COOPERATING UNITS (if any)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Gas liquid chromatography-mass spectrometry (GLC-MS), ion exchange chromatography, high pressure liquid chromatography (HPLC) and other separation procedures are used to isolate and measure the specific activities of the dopamine and norepinephrine (NE) metabolites, homovanillic acid (HVA), 3-methoxy, 4-hydroxyphenylglycol (MHPG) and vanillyl mandelic acid (VMA) in the urine of controls and patients with orthostatic hypotension as well as with various neurologic and mental disorders.

Deuterated D(-) MHPG has been injected intravenously into human controls quickly and as a slow, 3-hour infusion. Blood and plasma values for endogenous and deuterated MHPG and VMA have been analyzed to study the kinetics of conversion of MHPG to VMA and the rates of elimination of the compounds.

^{18}O dopamine has been injected into several human controls and the relative enrichments of several dopamine and norepinephrine metabolites studied.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

In humans, NE in the sympathetic nervous system and adrenal medulla is converted to VMA and MHPG. Small amounts (1-5%) of MHPG is in the free form and the rest is conjugated as the glucuronide or sulfate in approximately equal quantities. In brain, MHPG is the main metabolite of NE, with very small amounts of VMA being produced. Previous work in this and other laboratories suggest that MHPG in the free form is removed from brain directly to the blood.

Work we reported last year showed that free MHPG in the bloodstream is rapidly conjugated to the glucuronide or sulfate but free MHPG is converted to VMA. Approximately 50% of injected deuterated MHPG is converted to deuterated VMA. Thus, urinary VMA is largely derived from MHPG and urinary MHPG cannot reflect brain norepinephrine metabolism.

This year we have analyzed both plasma and urine, injecting the deuterated D(-)MHPG both quickly and using a 3-hour infusion. We have completed the analyses for endogenous and deuterated labelled free and conjugated MHPG and VMA. The results are consistent with rapid excretion of VMA formed largely (over half) from MHPG and slower excretion of MHPG conjugates. This work is now being prepared for publication.

PUBLICATION:

Blombery, P.A., Kopin, I.J., Gordon, E.K., Markey, S.P., and Ebert, M.H.: Conversion of MHPG to vanillylmandelic acid - implications for importance to urinary MHPG. Arch. Gen. Psychiatry 37: 1095-1098, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00272-10 LCS

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Metabolism, Distribution and Biochemical Effects of Psychoactive Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sanford P. Markey	Pharmacologist	LCS NIMH
Other:	Robert W. Colburn	Chemist	LCS NIMH
	Anthony P. Zavadil	Guest Worker	LCS NIMH
	Richard L. Hawks	Guest Worker	LCS NIMH
	Richard S. Burns	Clinical Associate	LCS NIMH
	Michael H. Ebert	Chief, Sect. on Exp. Ther.	LCS NIMH
	Irwin J. Kopin	Chief, LCS	LCS NIMH

COOPERATING UNITS (if any)

Sections on Medicine and Experimental Therapeutics, LCS, NIMH

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project transferred to Z01 MH 00351-06 LCS.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00274-07 LCS

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Methods of Ionization in Mass Spectrometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sanford P. Markey	Pharmacologist	LCS NIMH
Other:	Leonid Kellner	Visiting Scientist	BEIB DRS

COOPERATING UNITS (if any)

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1.5	1	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Alternate ion sources for enhanced sensitivity and performance of a quadrupole mass analyzer have been constructed and tested in both chemical and electron ionization modes. Lathanum hexaboride cathodes have been tested as alternative electron emitters for mass spectrometry.

PROJECT DESCRIPTION:

Previous success with the modification and application of a mass spectrometer for negative chemical ionization analysis has led to efforts to construct and test alternate ion sources which may have utility in biomedical research. Lathanum hexaboride polycrystalline directly heated cathodes were mounted on standard filament assemblies of Finnigan 3000 and LKB-9000 mass spectrometers. A cathode was made of a polycrystalline powder deposited on to an outgased 50 um thick carbon strip which was in turn mounted on metal holders (Ta). Typical parameters of operation were 2V, 1.5 to 2.3 A, and operating temperature around 1750° - 1800° K. Because of this lower operating temperature compared to tungsten or rhenium filaments, it is expected that the boride cathode will have an advantage when analyzing thermally unstable compounds. The cathode may have longer life than tungsten or rhenium filaments when operating at emission currents normally used in conventional ion sources (50 to 100 uA), will have lower power consumption, and could produce much larger emission currents (in excess of 10 mA). Comparison with regular filaments has been made in electron bombardment and chemical ionization ion sources in both positive and negative ion detection modes of operation. Long term studies are now in progress to determine whether the beneficial character of the boride cathode will withstand normal operating conditions. (See Project No. Z01 RS 10073-02 BEI for related work).

PUBLICATIONS

Markey, S.P.: Quantitative mass spectrometry. Biomed. Mass Spectrometry (in press), 1981.

Kellner, L., Fales, H.M., Markey, S.P. and Crawford, C.K.: Application of lathanum hexaboride cathodes in mass spectrometry. Proc. 29th Ann. Conf. Mass Spectrometry and Allied Topics (in press), 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00275-04 LCS

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Release and Turnover of Catecholamine Metabolites in Human Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief, LCS	LCS NIMH
OTHER:	Peter A. Blombery	Guest Worker	LCS NIMH
	Edna K. Gordon	Chemist	LCS NIMH
	Michael H. Ebert	Chief, Section on Expmtl. Ther.	LCS NIMH
	Jerry A. Oliver	Chemist	LCS NIMH
	Ronald Polinsky	Staff Physician	LCS NIMH
	Ib Oddershede	Visiting Fellow	LCS NIMH

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2.5	1.5	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

New methods have been developed using negative ion electron capture chemical ionization gas chromatography-mass spectrometry (EC/CI GC-MS) for assay of the norepinephrine (NE) metabolites, 3-methoxy-4-hydroxyphenylglycol (MHPG), dihydroxyphenylglycol (DHPG), normetanephrine (NMN) and metanephrine (MN). These methods increase the sensitivity of detection of these metabolites 50-100-fold over available electron impact (EI) GC-MS methods permitting the study of NE metabolism in much smaller amounts of biological material than was previously possible.

PROJECT DESCRIPTION:

Use of gas-chromatography-mass spectroscopy (GC-MS) has facilitated the development of sensitive and specific methods for assay of catecholamines metabolites in tissue, blood, and urine while simultaneously allowing determination of stable-isotopically labelled internal standards or products of administered labelled precursors.

Normetanephrine, metanephrine, and methoxydopamine in urine reflect secretion of norepinephrine, epinephrine, and dopamine, respectively. The amounts of normetanephrine appear to increase with age, corresponding to the higher plasma levels of norepinephrine in older subjects. Patients with autonomic insufficiency excrete lower than normal amounts of normetanephrine and MHPG. The relationship of the pattern of metabolite excretion to the type of autonomic insufficiency is being examined. This is also being studied in patients with hypertension, depression and after drug administration.

PUBLICATIONS:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00276-02 LCS

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Metabolism of Melatonin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sanford P. Markey	Pharmacologist	LCS NIMH
Other:	Masahiro Tetsuo	Visiting Fellow	LCS NIMH
	Alfred J. Lewy	Staff Psychiatrist	CPB NIMH
	Ronald Polinsky	Staff Physician	LCS NIMH
	Merrily A. Poth	Clinical Associate	IRP CH
	Philip L. Taylor	Visiting Fellow	LCS NIMH
	Irwin J. Kopin	Chief, LCS	LCS NIMH

COOPERATING UNITS (if any)
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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The pineal hormone melatonin and its major urinary metabolite 6-hydroxymelatonin are being quantified by gas chromatography-negative chemical ionization mass spectrometry. Assay of the urinary metabolite as an index of pineal gland activity has been validated by studies in monkeys on the effects of light and pinealectomy. Studies of the daily excretion rates of children have revealed that the pineal produces quantities of melatonin comparable to those of adults from the earliest ages measured (3 years). In boys, there is no significant change in daily 6-hydroxymelatonin excretion with respect to pubertal stage, but in girls, a significant elevation coincident with the onset of breast development was observed. Longitudinal studies are in progress to confirm these observations and relate them to changes in gonadotrophins. The precursor of melatonin, serotonin, has been quantified in monkey csf and marked diurnal variation observed.

PROJECT DESCRIPTION:

The function of the pineal gland in normal human physiology remains unknown. Lack of methodology to permit the measurement of the principal pineal product melatonin has been responsible for the absence of clinical studies on the role of this putative hormone. We have developed previously assays for the measurement of melatonin in plasma and its major urinary metabolite, conjugated 6-hydroxymelatonin. Plasma determinations of circulating hormone are useful to determine short-term changes in melatonin levels, such as those resulting from lighting or pharmacological manipulations. We have demonstrated that humans are responsive to light in that the normal nocturnal rise in melatonin levels can be reduced by exposure to light of daylight intensity (see Z01 MH 00450-05 CPB and Z01 MH 01851-03 CPB). Similarly, we tested the effects of light exposure on urinary excretion of 6-hydroxymelatonin by monkeys, and found that we could reduce excretion by 90% by constant exposure to 500 lux fluorescent lighting or by 96% by pinealectomy. Thus, 6-hydroxymelatonin is a valid index of its single precursor melatonin. The virtual disappearance of 6-hydroxymelatonin following pinealectomy precludes significant contribution of extrapineal melatonin, as has been previously postulated.

Studies on the effect of diminished β-adrenergic activity, such as that found in patients with orthostatic hypotension have been completed. Patients with significant peripheral sympathetic deficits exhibit low plasma norepinephrine levels and an inability to respond to mild stress, such as standing. In lower animals, the pineal is known to be noradrenergically innervated. Patient urines have been analyzed, and markedly diminished excretion of 6-hydroxymelatonin was observed in patients with peripheral sympathetic deficiency. Patients with central as well as peripheral involvement appear to exhibit a lack of diurnal rhythm or an uncoupled rhythm.

The role of melatonin in some species is antigonadotrophic, suggesting that it may have an important role in human reproductive biochemistry. Urinary metabolite studies in children at various stages of puberty were initiated in order to test this hypothesis. Urines were collected for consecutive two-day periods from 54 boys and 47 girls (ages 3-16) and 20 normal adults. No correlation exists between daily excretion rates and age in children ($12.9 \pm .6$ s.e.m. µg/day in boys, $15.9 \pm .8$ µg/day in girls) vs. adults ($11.4 \pm .75$). However, using Tanner criteria for pubertal stage, a significant increase in 6-hydroxymelatonin excretion was observed to be coincident with the onset of breast development in girls. Contrary to a report by others, prepubertal boys do not display elevated daily production of melatonin.

A negative cims assay for serotonin has been developed, analogous to the one used for melatonin. We have observed a marked circadian rhythm of serotonin in monkey csf, and are currently determining the effects of light, sleep, and drugs on this rhythm.

PUBLICATIONS:

Tetsuo, M., Markey, S.P., and Kopin, I.J.: Measurement of 6-hydroxymelatonin in human urine and its diurnal variations. Life Sci. 27: 105-109, 1980.

Tetsuo, M., Polinsky, R.J., Markey, S.P., and Kopin, I.J.: Urinary 6-hydroxymelatonin excretion in patients with orthostatic hypotension. J. Clin. Endocrinol. Metab. (in press), 1981.

Markey, S.P., Colburn, R.W., and Johannessen, J.N.: Efficient extraction and mass spectrometric assay of serotonin in biological fluids. Biomed. Mass Spectrom. (in press), 1981.

Lewy, A.J., Wehr, T.A., Goodwin, F.K., Newsome, D.A., and Markey, S.P.: Light suppresses melatonin secretion in humans. Science 210: 1267-1269, 1980.

Tetsuo, M., Markey, S.P., Colburn, R.W., and Kopin, I.J.: Quantitative analysis of 6-hydroxymelatonin in human urine by gas chromatography-negative chemical ionization mass spectrometry. Anal. Biochem. 110: 208-215, 1981.

Tetsuo, M., Perlow, M.J., Mishkin, M., and Markey, S.P.: Light exposure reduces and pinealectomy virtually stops urinary excretion of 6-hydroxymelatonin by rhesus monkeys. Endocrinology (in press), 1981.

Tetsuo, M. and Markey, S.P.: Quantification of 6-hydroxymelatonin by gc-negative cims. Proc. 28th Ann. Conf. Mass Spectrometry and Allied Topics: 282-283, 1980.

Taylor, P.L. and Markey, S.P.: High resolution gas chromatography-negative chemical ionization mass spectrometry of indole amines. Proc. 29th Ann. Conf. Mass Spectrometry and Allied Topics (in press), 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00277-02 LCS

PERIOD COVERED
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Synthesis of Stable Isotope Labelled Compounds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sanford P. Markey	Pharmacologist	LCS NIMH
Other:	Wilfried J.W. Mayer	Visiting Fellow	LCS NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Synthesis of oxygen-18 and deuterium labelled compounds are being developed for clinical studies on their metabolic fate and for internal standards in mass spectral assays. General synthetic reactions are being studied in an effort to provide key labelled intermediates in high yield. Synthesis of di-oxygen-18 labelled 3,4 dihydroxyphenylethyleneglycol (DHPG) has been completed on a test scale.

PROJECT DESCRIPTION:

Stable isotope analogs or isotopimers of biogenic amines are being used for human clinical research (Z01 MH 00275-04 LCS) to determine the metabolism and kinetics of distribution and excretion of neurotransmitters. Common to many of the compounds of interest are catechol oxygens, generally enzymatically monomethylated during metabolism. Thus, routes of synthesis of oxygen-18 labelled catechols or mono-deuteromethylated catechols are being pursued.

Using the previously described (MH 00277-01) route of synthesis of veratrole - $^{18}O_2$ from 0-difluorobenzene, the norepinephrine metabolite 3,4 dihydroxy-$^{18}O_2$ - phenylethylene-glycol has been prepared in low milligram amounts. Veratrole-$^{18}O_2$ demethylated to catechol; mono-benzylated, condensed with glyoxalic acid, debenzylated and reduced to the desired product. Current efforts are to produce sufficient material of high isotopic purity to permit animal and human pharmacokinetic studies.

PUBLICATIONS:

Markey, S.P., Mayer, W.J.W., and Oliver, J: Synthesis of stable isotope analogs of biogenic amines. Proc. 28th Ann. Conf. Mass Spectrometry and Allied Topics: 519-520, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00245-05 LDP

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Objective Identification of the Hyperkinetic Syndrome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Thomas M. Achenbach	Research Psychologist	LDP	NIMH
Other	Gerald L. Brown	Research Psychiatrist	LDP	NIMH

COOPERATING UNITS (if any)
Biological Psychiatry Branch, NIMH

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, MD 20205

Total Staffyears:	PROFESSIONAL:	OTHER:
.00	.00	.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the study is to identify objectively the behavioral patterns associated with findings obtained through intensive medical workups of children referred for hyperactivity. Both parents of a referred child independently complete a behavior checklist from which a standardized behavior profile is derived. Degree of interparent agreement is computed and the profiles are analyzed in order to classify children according to types of behavior patterns. Classification of children in this way is then compared with ratings of ward behavior, with the outcomes of the medical work-up, and with the effects of stimulant drugs to determine whether the behavioral typology corresponds to characteristics of the children identified clinically.

PHS-6040
(Rev. 10-76)

Project Description

The "hyperkinetic syndrome" is today the most widely publicized of childhood disorders. There has been a tremendous increase in the number of children labelled as hyperkinetic and a great deal of controversy over such diverse approaches to treatment as chemotherapy, dietary manipulations, behavior therapy, and laissez faire permissiveness. Etiological hypotheses show a parallel diversity, with minimal cerebral dysfunction, allergies, anxiety, inappropriate reinforcers, constitutional characteristics of temperament, and creative nonconformity all being implicated by one authority or another.

One reason for the diversity of treatment and etiological hypotheses is that there is no objective way to define the behavioral phenotype of hyperkinesis. Since most children are from time to time too active for the tastes of their parents and teachers, and since the terms "hyperactivity" and "hyperkinesis" have gained such wide currency, reports of high activity level alone cannot be regarded as pathognomic. Identifying children who share some common etiological factor with respect to activity level or who would benefit from a particular treatment has thus been made difficult by the excessively broad application of the concept of hyperkinesis.

In order to determine whether there is an identifiable behavioral phenotype corresponding to a clinical syndrome which may include organic anomalies and which may benefit from chemotherapy, collaborative research is being carried out with Dr. G. L. Brown of the NIMH Biological Psychiatry Branch in connection with a study of children referred for hyperactivity (cf. Biological Psychiatry Branch Project Number Z01 MH 0100-04BP). Both parents of each referred child independently complete the Child Behavior Checklist (described in report on Project Number Z01 MH 00144-03 LDP). An extensive life history is also obtained and the children are admitted to the NIH Clinical Center for several weeks of medical, behavioral, and pharmacological assessment. Without knowledge of the results of these assessments, we are scoring the behavior profile patterns from the Checklist completed by each parent. Interparent reliability is assessed and the separate profiles as well as a composite profile based on the checklists completed by both parents are analyzed to identify particular types of profile patterns. These are being compared with the results of the clinical work-ups to determine whether the types of profile patterns correlate with differential clinical findings and with ratings of ward behavior. Preliminary findings indicate a clearcut behavioral phenotype of hyperactivity and high correlations between parent ratings on our Checklist and one developed by C. Keith Conners for use in evaluating the effectiveness of drug treatments with children.

If further relationships between the behavior profile patterns and clinical findings are confirmed, the profile patterns can be employed to form homogeneous groups for research purposes. As specific etiologies and/or treatments are discovered for these groups, the profile patterns can be employed by clinicians to guide diagnosis and the choice of treatment.

This project has been terminated because the PI is no longer at NIMH. This is a final report.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00246-06 LDP

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

A Follow-up Study of Children Differing in Behavior Problems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Thomas M. Achenbach Research Psychologist LDP NIMH

COOPERATING UNITS (if any)

3 Child Guidance Clinics in New Haven and Waterbury, Connecticut

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

Total Staffyears:	PROFESSIONAL:	OTHER:
.00	.00	.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the research are (1) to determine the stability of behavior profiles based on behavior checklists describing disturbed children, and (2) to determine the prognosis for children differing in initial profile patterns. Parents of children, for whom Child Behavior Checklists were filled out at intake, tilled out Checklists describing their children's behavior problems and social competencies following treatment. Behavior profiles based on the follow-up Checklists are being compared with those based on the initial Checklists to determine the stability and differential prognosis for different types of profiles in various age, sex, and socio-economic groups.

PHS-6040
(Rev. 2-81)

Project Description:

Effective utilization of mental health services to children is greatly handicaped by the current lack of knowledge about prognosis for various behavior problems. Although the demand for services to children far exceeds the supply, there is no objective basis for selectively assigning treatment to those who will benefit most, because it is not known which children will actually be helped by treatment, which ones will improve without treatment, and which ones will fail to be helped by current treatment approaches.

A primary obstacle to research on prognosis has been the lack of a standardized and reliable diagnostic-classification system for childhood behavior disorders. As described in the report on Project Number Z01 MH 00244-05 LDP, the investigators have developed a standardized behavior profile for child psychopathology and behavioral competence. It is expected that children having different profile patterns will differ in their prognosis under various conditions of treatment and nontreatment.

To test both the stability and the predictive power of the profile patterns, a follow-up was conducted of 200 children for whom Child Behavior Checklists were filled out in three clinics at intake. Parents of these children were asked to fill out Checklists describing their children's behavior problems and competencies following termination with the clinics. Statistical analyses have shown that children who differed in their initial profiles subsequently differ with respect both to their patterns of problems and competencies and to their general adjustment. The findings also indicate statistically significant stability in the patterns of reported problems despite significant declines in the number of problems reported.

Further analyses are expected to show whether initial profile patterns are differentially related to treatment outcome and whether the patterns are useful for determining prognosis under typical clinic procedures. The data will also be compared with outcome data for children seen in other settings and those receiving systematic assignment to particular treatments.

The data obtained in Project Z01 MH 00269-03 were included in this project.

This project has been terminated because the PI is no longer at NIMH. This is a final report.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00247-06

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Patterns of Psychological Functioning in Children with Endocrine Abnormalities

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jerome H. Blue	Staff Fellow	LDP NIMH
OTHER:	Thomas M. Achenbach, Ph.D.	Research Psychologist	Univ. of Vermont Burlington, Vt.
	Craig Edelbrock, Ph.D.	Research Psychologist	Boys Town, Ne.
	Roger Johnsonbaugh, M.D.	Pediatric Endocrinologist	National Naval Medical Center

COOPERATING UNITS (if any)
University of Vermont, Burlington, Vermont
Boys Town, Nebraska
National Naval Medical Center, Bethesda, Maryland

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
.31	.22	.09

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Standardized assessments of children's behavior problems, competencies and cognitive functioning are being obtained on normal controls and children who have thyroid and sex hormone abnormalities in order to determine whether hormone levels affect patterns of cognitive abilities and behavioral adaptation. The study is designed to test the hypothesis that increases in particular sex hormone levels affect patterns of cognitive functioning by improving automatization abilities at the expense of ability to engage in complex perceptual restructuring. It is also designed to determine whether premature craniosynostonis noted in hyperthyroid children interferes with cognitive functioning.

PHS-6040
(Rev. 10-76)

Project Description:

Research and theory tend to focus on cognitive disabilities as one substantive area and "emotional" problems as a separate substantive area. However, from a developmental perspective, cognitive functioning is intimately inter-woven with the social, biological, and emotional aspects of adaptive processes. On the one hand, a child's level of cognitive development is likely to set limits on the types of adaptive strategies available to him or her. On the other hand, social, biological, emotional, and motivational factors are likely to influence the degree to which children make use of their cognitive capacity. The focus of this project is to examine the interface between cognitive functioning and maladaptive behavior.

Work by other investigators has suggested that sex hormone levels affect the action of neurotransmitters in the brain, and that these, in turn, affect the patterning of cognitive abilities. In particular, it has been found that high levels of sex hormones, especially estrogens, are positively correlated with performance on automatization tasks requiring quick, accurate responses, but are negatively correlated with performance on tasks that require complex perceptual restructuring. Our study is designed to test this hypothesis by comparing cognitive functioning and behavioral patterning before and after treatment in children who receive sex hormone supplementation for various endocrine abnormalities. Children with thyroid abnormalities are also being studied because clinical observations have revealed premature craniosynostonis in these children, and it is important to determine whether cognitive and behavioral patterns are affected by this condition.

Measures of cognitive functioning include subtests of the Wechsler Intelligence Scale for Children (WISC), the Embedded Figures Test, Color naming and and Object naming test. Differences between perceptual restructuring measures (i.e., WISC Block Design, Embedded Figures Test) and automatization measures (i.e., Colornaming, Object-naming) will be compared at two testing sessions to determine whether changes in hormone levels after medication cause changes in cognitive patterning that do not occur in normal, unmedicated control participants. The child's favorite activities, school performance, and areas of behavioral strength and weaknesses will be examined using interview protocol Form No. ADM512 OMB No. 68. S75110.

This protocol has only recently been reactivated and at present there is not enough data to draw cnclusions about the effects of sex hormones and/or the relationship between hormones and behavioral variables.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00255-05 LDP

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Maternal Imprisonment: Psychological Effects on Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Velma LaPoint	Staff Fellow	LDP	NIMH
Other:	Marian R. Yarrow	Chief	LDP	NIMH

COOPERATING UNITS (if any)

Maryland Division of Corrections, Baltimore, Maryland
Maryland Division of Parole and Probation, Baltimore, Maryland
National Institute of Justice, (LEAA), Department of Justice, Washington, D.C.

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

Total Staffyears:	PROFESSIONAL:	OTHER:
.10	0	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The lack and/or loss of stable and supportive parenting when a mother is imprisioned represents a psychological at risk situation for children. The purpose of the study is to identify psychological consequences for children whose mothers are imprisioned. Risk for children is compounded when the reasons for strained, inadequate parenting are themselves indicative of psychological problems and stress of mothers. Research participants are imprisoned mothers, their children, and children's caregivers. Mothers and children from single-parented families in the same community comprise the comparison groups: mothers who are on probation, and mothers with no previous involvement in the criminal justice system. The groups are comparable racially (Black) and socioeconomically. Data were obtained in home contacts, mother-discussion groups, mother-child dyads and group interactions. Secondary data were obtained from school records.

PHS-6040
(Rev. 10-76)

Project Description:

The loss of stable and supportive parenting places children at risk for mental health problems. Risk for the child is compounded when the reasons for inadequate parenting are themselves indicative of psychological problems of the parents. Children whose mothers are imprisoned constitute such a group at risk. The purpose of this study is to identify psychological correlates and consequences for children of maternal imprisonment. Relationships of mother, child, and caregiver, the child's experiences in the process of mother's incarceration, and the child's problems and his/her abilities to cope with daily demands of living are investigated. An examination of the child's supportive network is included.

Research participants from Baltimore, Maryland are 41 imprisoned mothers at the Maryland Correctional Institution for Women, Jessup, Maryland and their children who reside with relatives in their homes in Baltimore. Children are between 5 and 12 years old. Mothers and their children, from single parented families, comprise two comparison groups: mothers on probation, and mothers with no previous involvement in the criminal justice system.

Data were obtained in (a) home contacts, (b) mother discussion groups, (c) mother-child dyads, and (d) group interactions. A secondary data source is school records of children's academic and behavioral competence. The prison setting was utilized when data collection related to incarcerated mothers in discussion groups or with their children. A community (church) setting was used for other data collection.

Maternal data were obtained on mother's roles and attitudes toward their children and attitudes toward themselves as mothers. Assessments were made of mother-child relationships, child affective states, attitudes toward self and adult authority figures, and problems and competencies in coping with day-to-day demands.

Findings:

Preliminary findings have been presented in previous Annual Reports. Papers are in preparation and/or under editorial review. A prinicipal investigator who is no longer at the Laboratory will continue the analyses and the preparation of scientific reports.

This is a final report.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00257-05

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Effects of CNS Treatment on Intellectual Functioning of Children with Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard A. Moss	Research Psychologist	LDP NIMH
OTHER:	David G. Poplack	Senior Investigator	POB NCI
	Judith Rumsey	Staff Fellow	BP NIMH
	Pim Brouwers	Visiting Fellow	CN N

COOPERATING UNITS (if any) Various Children's Hospitals throughout the United States
National Cancer Institute
Clinical Neurosciences Branch, N
Biological Psychiatry Branch, NIMH

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
.74	.47	.27

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of CNS treatment (cranial irradiation and intrathecal chemotherapy) on the cognitive and perceptual functioning of children with leukemia are investigated. One study compared a group of leukemic children in remission with a matched group of sibs on measures from a battery of psychological tests (Wechsler, Bender-Gestalt). These groups are followed-up 12-15 months after the initial assessment. Non-CNS treated comparison groups are also being assessed using these procedures to control for effects other than CNS treatment. A second study deals with another sample studied prospectively. In this instance children in remission from leukemia are evaluated on the same battery of psychological tests as in the first study. They are tested just prior to prophylactic CNS treatment and are re-evaluated one year later to determine if any possible brain changes brought on by the CNS treatment have resulted in learning disabilities and diminished intellectual functioning. A five year longitudinal study investigating the neuropsychological correlates of CNS treatment of over 180 leukemic cases at various Children's Hospitals is being initiated.

PHS-6040
(Rev. 10-76)

Project Description

The chance of survival from childhood leukemia has increased greatly over the past 15 years because of the utilization of new treatment procedures. The main change in treatment that has led to this improved outlook is the inclusion of prophylactic measures consisting of a series of cranial irradiations and intrathecal (spinal) injections of powerful chemical agents.

The purpose of the proposed research is to determine if CNS treatment has deleterious effects on intellectual functioning and to evaluate the nature and extent of these effects. Two studies are in progress. One study involves evaluation of intellectual functioning only after CNS treatment (post-treatment study); in the other study intellectual performance prior to CNS treatment is compared to performance subsequent to treatment.

Because of the age range of our subjects, the three Wechsler Intelligence Tests are used: WPPSI (ages 4-6), WISC-R (ages 6-16), WAIS (17 years of age and over).

Study I. Post-treatment study.

The sample consists of 34 children who have been in remission for 2 to 5-1/2 years from acute lymphocytic leukemia (ALL), and a sibling control group. The mean age of the children at the time of assessment was 11 years with a range of 4 to 23. Thirteen leukemia patients who were treated for their leukemia without the use of CNS treatment and their siblings, and 16 cystic fibrosis patients and their siblings served as control groups. All children were administered a Wechsler Intelligence Test and the Bender-Gestalt.

For most comparisons involving the central nervous system treated group, the siblings performed at a significantly higher level of functioning than the patients. The mean Full Scale IQ of 99 for patients and 113 for siblings at an initial testing and 100 and 116 respectively, at a one year follow-up testing indicates that the patients are operating at an average and the siblings at a bright average level of intellectual functioning. There were no IQ differences between the patients and the siblings for the non-CNS treated patient group.

As previously reported, these data have shown that the longer the time interval between central nervous system treatment and the psychological assessment the greater the IQ decrement, and the younger the child at the time treatment was initiated the greater the apparent IQ loss. The variables of "time interval between CNS treatment and assessment" and "age of the child at the time treatment was started" were combined, using a ratio, into a new variable which reflected the "percentage of life lived since the initiation of treatment." This "percentage of life lived since treatment" variable was even more strongly related to IQ decrement than the previous two separate variables. There was also evidence that brighter children were significantly more affected by the CNS treatment than the children of average potential. A measure of variability among Wechsler subtest scores (scatter), which has

been used as a diagnostic index of pathology, showed significantly more "scatter" for the CNS treated ALL patients.

Analyses are currently under way to try to develop diagnostic indices concerning brain damage from the Wechsler tests using the CNS treated patients as a criterion group.

Study II. Prospective Study.

A sample of 60 children with acute lymphocytic leukemia, who receive prophylactic CNS treatment, are being administered the same psychological evaluation described in the Post-treatment study. This assessment is made before CNS treatment and one year after treatment. This interval of one year should be sufficient to allow for loss in learning abilities if such losses occur as the result of treatment. The sibling group of leukemic patients receiving CNS treatment who were a part of the Post-treatment study will be used as a control group for this study to partial out any practice effects that may contribute to the follow-up IQ scores among the patients.

Half of this sample will be between 3-1/2 and 6 years of age and half will be between 6 and 16 years of age. This age distribution will provide a good basis for evaluating whether age at the time of CNS treatment is a factor in contributing to the degree of intellectual deficit that may occur. Initial testing has been done in about 45 cases and the one year follow-up in about 25 cases.

Study III. Collaborative Prospective Study Involving Different Medical Treatments.

A large scale clinical study has been initiated under the direction of the National Cancer Institute and include patients from Children's Hospitals in Seattle, Washington, Northern California (Oakland), Columbus, Ohio, The District of Columbia, and the Pediatric Oncology service of the National Cancer Institute. The purpose of this research is to compare the relative effectiveness and the degree of neurotoxicity associated with treating a sample of 180 acute lymphoblastic leukemia (ALL) patients with either cranial irradiation plus intrathecal methotrexate or with high dosage systemic methotrexate infusions alone. It is known that cranial irradiation and intrathecal methotrexate produce structural changes in the brain and we have also established that this treatment results in lowered intellectual functioning. It is unknown what effects the high dosage infusion methotrexate has on the central nervous system. The psychological aspects of this research will consist of intellectual, neuropsychological, behavioral, and personality assessment and comparisons between these two treatment groups. The sample will consist of children from 1 to 21 years of age who will be evaluated annually for a five to seven year period. The first assessment will be made after remission is brought about from standard chemotherapy but prior to initiation of the central nervous system or high dosage infusion methotrexate treatment. The Stanford-

Binet, McCarthy, and Wechsler scales will be administered, in terms of their age appropriateness, for measuring intellectual functioning. A series of neuropsychological procedures are being selected and designed for measuring those brain functions that would appear to be most likely to be impaired by the CNS treatment. These functions are attention, new learning, problem solving, immediate and delayed memory, academic achievement, and sensory and tactile motor functioning. Deficits on these abilities can be helpful in identifying the localization of brain impairment. Since the total brain is exposed to the CNS treatment, the neuropsychological tests might be able to indicate whether different portions of the brain were differentially vulnerable to the neurotoxic stimuli. Thus, such research could enhance our knowledge about brain physiology and differential resilience of various brain structures. The Achenbach Behavior Checklist will be used for measuring whether social competence and behavioral adaptiveness are effected by experiencing the illness and from the treatments. A modification of the Block Child Personality Q-Sort procedure will be administered to the mothers of these children to determine if personality characteristics tend to be altered. In addition to including 57 of the standard Block items this Q-Sort will include items from the Jackson Personality Inventory, Locus of Control, helplessness-Mastery, and Cautiousness scales. This research will also include two control groups: a group of 40 matched siblings and a group of 40 children with solid tumors who do not receive any treatment directed at the central nervous system. These control groups will be tested twice at a yearly interval and will be selected so as to be representative of the patient sample in terms of age, ordinal position, and socioeconomic status. Our Laboratory has planned the research, in collaboration with neuropsychologists at NIH and the various participating institutions, and will coordinate the collection and analyses of the psychological and neuropsychological data.

Publications:

Moss, H. A., and Nannis, E. D. Psychological Effects of Central Nervous System (CNS) Treatment of Children with Acute Lymphocytic Leukemia (ALL). In Kellerman, J. (Ed.): Psychosocial Aspects of Childhood Cancer. Springfield, Charles C. Thomas, Publisher, 1980, pp. 171-183.

Moss, H. A., and Susman, E. J. Longitudinal Study of Personality Development. In Kagan, J. and Brim, O. (Eds.), Constancy and Change in Human Development: A Volume of Review Essays. Cambridge, Mass., Harvard University Press, 1980, pp. 530-595.

Moss, H. A., Nannis, E. D., and Poplack, D. G. The Effects of Prophylactic Treatment of the Central Nervous System on the Intellectual Functioning of Children With Acute Lymphocytic Leukemia. Am. J. Med., in press.

Appendix to Project Number Z01 MH 00257-05

Contract # 263-MD-001195 for $1480 has been used to reimburse testers, $40 per case. The tests given are the WPPSI, WISC, WAIS and Bender-Gestalt. The work involved is the equivalent of 1/20 man years.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00259-05

PERIOD COVERED
October 30, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)
Relations Between Parent, Teacher and Clinician Reports of Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Thomas M. Achenbach Research Psychologist LDP, NIMH

COOPERATING UNITS (if any)

Woodburn Center for Community Mental Health, Annandale, Virginia

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.00	.00	.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the study are (1) to determine the relationships between parent, teacher, and clinician reports of the behavioral problems and competencies of children referred for outpatient mental health services; (2) to determine the relationship between children's presenting behavioral patterns as reported by these sources and the outcome of their clinic contacts. At intake, and again following termination with the clinic, parents and a clinician filled out the Child Behavior Checklist, while the child's teacher filled out a Teacher Report form. Child Behavior Profiles scored from these data have been compared to identify agreements and disagreements between parents, teachers, and the clinician. The relationship of outcome to intake Profile patterns, demographic variables, and other characteristics of the children will also be assessed in order to identify predictors of poor outcome. If children who typically have poor outcomes can be identified, new treatment strategies may be developed to help them.

Project Description:

Little is known about the relationships between client characteristics and the effectiveness of existing treatments for child psychopathology. One of the purposes of the Child Behavior Profile (Project No. Z01 MH 00244-05 LDP) is to differentiate among children who differ in their responses to various treatment modalities. This study is designed to determine the degree of agreement and the nature of disagreements between Profiles scored from Child Behavior Checklists completed by children's parents, their teachers, and a clinician who did an intake and follow-up evaluation but was not involved in treatment. It is also designed to determine whether classification of children according to Profile patterns derived from reports by each type of informant separetely and/or in composite is predictive of outcome in response to the child mental health services offered by a community mental health center.

In addition to the Behavior Profiles, the intake clinician obtained data on family composition, demographic characteristics, previous mental health contacts, degree of parent motivation for help, and the problems considered most serious by the partents. Six months after intake into the clinic, the intake clinician contacted the family to obtain Behavior Cheklists describing the child's current status, plus data on changes in the family, the family's reactions to the services they have received, and possible needs for further services.

If children who have poor outcomes can be discriminated from those who have good outcomes on the basis of intake data, then new treatment strategies can be developed for helping children who are not likely to benefit from mental health services as currently offered. Initial analyses show correlations averaging about .80 between Child Behavior Profile scores from Checklists filled out by the clinician and the children's mothers at intake and about .75 at follow-up; correlations averaging about .41 between the clinician's ratings and teachers' ratings at intake and about .51 at follow-up; and coorralations averaging about .28 between teachers' ratings and mothers' ratings at intake and negligible correlations at follow-up, Categorization of Profiles in terms of cluster-analytically based typologies has shown agreement ranging 70 to 95% in profile patterns scored independently from Checklists filled out by the clinician and athe children's mothers. Further analyses will be made to identify the best predictors of outcome as judged by parents, teachers, and the clinician.

This project has been terminated because the PI is no longer at NIMH. This is a final report.

Publications:

NONE

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00265-05 LDP

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Chronic Malnutrition and Child Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David E. Barrett	Research Psychologist	LDP	NIMH
Other:	Marian Radke-Yarrow	Chief	LDP	NIMH

COOPERATING UNITS (if any)

Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

Total Staffyears:	PROFESSIONAL:	OTHER:
.47	.10	.37

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Behavioral effects of chronic undernutrition were investigated. Subjects were 138 children, age 6-8, in rural Guatemala who had participated in the INCAP Longitudinal Study. Pre- and postnatal intakes of calorie supplements were the independent variables. Dependent measures were assessments of social interaction and affect. These measures were obtained by observing children in smallgroup activities with peers, and from individual cognitive tests. High calorie supplementation from birth to 2 years predicted high levels of social involvement and both happy and angry affect at school-age, as well as moderate activity level, and low levels of dependency on adults, passive behavior, and anxious behavior. Relations were significant with SES and maternal supplementation controlled. Except for attentional measures, cognitive measures were not strongly predicted by supplement intake.

PHS-6040
(Rev. 10-76)

Project Description:

The present study is concerned with the effects of chronic malnutrition on behavioral competence at school-age. Two related areas of investigation provide a theoretical base. Research on animals with a history of undernutrition identifies a consistent pattern of behavioral abnormalities which includes apathy, passivity, and attentional impairments, reduced exploration and avoidance of new stimuli and abnormal responses to social initiations. Recent studies of malnourished human infants reveal similar behavioral characteristics: reduced social responsiveness, low activity level, and little affect. Such tendencies, if they continued to characterize development, would be expected to impair the quality of the organism's social interactions and affect, and inhibit the development of social competencies.

The behavioral effects of chronic malnutrition were investigated in this study by analyzing relationships between pre- and postnatal nutrient intake and social behavior, affect, and cognitive functioning at school-age. Subjects were 138 children, ages six to eight, from three villages in eastern Guatemala. All had participated in the INCAP (Institute of Nutrition of Central America and Panama) Longitudinal Study (1969-1977) in which ad libitum calorie supplements had been provided to pregnant women and later to their children. INCAP investigators have consistently found that supplement calorie intake was the best predictor of postnatal physical growth and cognitive development, relative to other nutritional (e.g., diet protein or calories) and social-environmental variables. For these reasons, supplement calorie intake measures were the primary independent variables. Three measures were used: (a) mother supplement calories during pregnancy, (b) child supplement calories from birth to two years, and (c) child supplement calories from two to four years. To ensure that supplement effects were not confounded with pre-existing village differences, analyses were carried out both across and within villages. A composite measure of social environment (quality of house, parents' clothing, and maternal stimulation of the child) was included as a covariate in the analyses.

Dependent measures were assessments of the child's interaction with peers and adults, emotional characteristics, and intellectual development. Children were systematically observed, in small play groups by experienced Guatamalan observers, in two 2 1/2-hour sessions. The sessions were comprised of a sequence of activities that included peer interaction in a novel environment, problem-solving tasks, competitive activities, and impulse-control situations.

Individual testing was also done: Measures, standardized by INCAP, were Verbal Inferences, Hidden Figures, Concept Identification, Block Design, Memory for Objects, and Digit Span. The Bender Gestalt, a test of persistence/distractability on an impossible puzzle, and a test for following directions after a period of delay were administered.

Multiple regression analyses showed that calorie supplementation from birth to two years, with SES and mother supplementation controlled, was significantly associated with high levels of social involvement, both happy and angry affect, and moderate activity level ($p<.01$); low levels of passive behavior and anxious expression ($p<.01$); and low dependency on adults ($p<.05$). Supplementation from two to four years was generally significantly associated with the same variables, but did not increase the variance accounted for in these measures when earlier child supplementation (birth to two years) was in the equation. Similar findings were obtained within villages.

Cognitive abilities were not strongly predicted by supplement intake.

Addendum

This project was carried out by the Laboratory staff in collaboration with INCAP. A contract of $25,000 from November 1978 to November 1979 was used to train personnel from local areas and to support them for the collection of data. A member of the Laboratory staff was involved in the field work. The data analysis, writing, and editing of the paper has been performed under a Professional Services Contact which covers the period October 1, 1980 to September 30, 1981.

Publications:

Barrett, D. E. & Yarrow, M. R. Effects of early malnutrition on social behavior at school-age. Pediatric Research, 1981, 15, 445.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02132-04 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Children's Misdeeds, Parental Discipline, and Children's Compliance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
Other:	Michael Chapman	Staff Fellow	LDP	NIMH
	Leon Kuczynski	Visiting Fellow	LDP	NIMH

COOPERATING UNITS (if any)

Laboratory of Developmental Psychology

LAB/BRANCH

SECTION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

INSTITUTE AND LOCATION

TOTAL staff years:	PROFESSIONAL:	OTHER:
.43	.24	.19

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Effects of children's misbehaviors and parents' disciplinary methods on children's compliance and internalization of rules were studied. Twenty-four mothers of one-to-two year old children were trained to report incidents of children's affective interactions, from which sequences of children's misbehaviors, maternal discipline and children's subsequent compliance or noncompliance were extracted. Parallel measures were obtained five years later and, in addition, experimental measures of child compliance and adherence to rules were obtained. Both past and current parent and child behaviors will be examined in regression equations to determine the best predictors of children's compliance and internalization.

PHS-6040
(Rev. 10-76)

Recent conceptualizations of children's personality development in relation to parental socialization practices highlight the influences that both parent and child have on the outcome of parents' discipline on children's compliance. The purpose of this research is to study, longitudinally, the interaction of children's misbehaviors with parents' disciplinary practices, in terms of their cumulative influences on children's compliance and internalization of rules. Several specific questions are addressed. What are the precursors of disciplinary practices? What are the outcomes of different discipline practices on children's compliance and noncompliance? What are the developmental changes in patterns of discipline and compliance?

Twenty-four middle class mothers and their children were studied for nine months when the children were one-to-two years old and were seen again for three months, five years later. Mothers were taught to observe and report natural incidents of children's interactions involving emotions. From these reports, sequences of children's misbehaviors, mothers' discipline practices, and children's compliance or noncompliance were obtained. Ratings of mother and child were made during home visits. Experimental measures of children's compliance and adherence to rules, and measures of compliance to mother's requests in standard situations were obtained at the five-year follow-up. Reliability checks on mothers' reporting skills were also assessed in the laboratory sessions.

In the description of findings reported last year, parent-child interaction, when the children were one- to two-years old, forms of parent discipline and child misbehaviors were identified and associations described. Misbehaviors involved interpersonal aggression, property harm, and problems with impulse control. Mothers' techniques of discipline were coded as explanation, instruction, verbal prohibition, physical punishment, physical restraint, withdrawal of love, and dramatization of distress. Certain discipline practices predictably followed certain misbehaviors. Love withdrawal, in combination with other disciplines, was the form of discipline most likely to result in compliance. The next research question concerns whether similar patterns continue to prevail or whether additional, different predictors of compliance emerge in the later developmental period studied.

One manuscript is under review and another manuscript has just been submitted.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 MH 02135-04 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Emotional Development in Children of Depressed Parents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
Other:	Marian Radke-Yarrow	Chief	LDP	NIMH
	Mark Cummings	Staff Fellow	LDP	NIMH
	Ronald Iannotti	Intergovernmental Personnel Act Fellow	LDP	NIMH
	Leon Cytryn	Research Psychiatrist	BPB	NIMH
	Donald McKnew	Research Psychiatrist	BPB	NIMH

COOPERATING UNITS (if any)

Laboratory of Biological Psychiatry, NIMH
Laboratory of Psychobiology, NIMH

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staffyears:	PROFESSIONAL:	OTHER:
.85	.32	.53

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The emotional development of young children in families with parental psychpathology is examined. Families are those in which one parent has a history of bipolar affective illness or in which there is no diagnosed parental psychopathology. Children 10 to 12 months old are followed longitudinally. Observations of children's responses to emotional events in the familial environment are obtained from trained parent observers and are also videotaped during home visits. In laboratory sessions, children's positive and negative peer interactions are assessed. Children are also diagnosed for psychopathology. Children's responses to emotions, both the transient and enduring distress states of others, provide information about how children develop and maintain adaptive and maladaptive patterns of coping with environmental stresses.

PHS-6040
(Rev. 10-76)

Project Description:

The focus of this research is on the affective development of children in families where one parent has a manic-depressive disorder. One major dimension of interest is children's intrapersonal functioning, i.e., their empathic, aggressive and avoidance responses. The development of psychopathology in children from these families is also assessed. The familial recurrence of manic-depressive illness across generations has been documented in research. Further, some studies suggest a severe impact of the parent's illness on the psychosocial adaptation of school-age children. While there is evidence to indicate a genetic predisposition for bipolar affective illness, the aberrant affective environments, also may be an integral part of the transmission process. The high levels of stress and distress that characterize manic-depressive families, the unpredictibility of these parental personalities, and the unusual childrearing practices of these parents may place the children at risk. These affective environments and their impact on children are assessed in a longitudinal investigation.

Seven children with a bipolar parent and 30 control children from normal families were studied. A subsample of the control group were children whose parents had been screened and selected on the basis of total absence of psychopathology in the immediate family. Thus, different control groups were used, representing different degrees of normalcy. Children entered the study at one year of age and have been followed for two years. Data were obtained from multiple respondents (e.g., mothers, clinicians) in naturalistic and experimental settings. (1) Mothers were trained to report children's responses to positive and negative emotions occurring in the natural environments, as well as children's responses to simulations of emotions. (2) Children's reactions to simulations of distress emotions (e.g., sorrow, depression, pain) were videotaped during home visits. (3) Tests of inferential skills (object permanence and self-other differentiation) were done in home visits during the second year of life. (4) At 24-30 months, children's interactions with peers were examined in three laboratory sessions intended to elicit individual differences in patterns in social adjustment. (5) Throughout the study, home visitors made global assessments of symptomatology (e.g., fears, sleep disturbances, eating problems). Parental rearing practices and discipline techniques, and the explicit communication practices that parents use to teach their children about emotions were also assessed. At the age of three, children will be screened for signs of psychopathology.

Data collection is near completion. Preliminary analyses indicate symptoms of disturbance in many of the target children of bipolar patients and in their siblings as well (e.g., hyperactivity, passivity, sleeping problem, etc.). Also documented is the early emergence in some children of bipolar parents of stable styles of coping with others' stresses and distresses. For example, one pattern is heightened empathy and or hypersensitivity toward victims in distress; another pattern is systematic avoidance and withdrawal. Coding and data analyses will continue throughout the year.

Publications:

Zahn-Waxler, C. Maternal Child Rearing Practices in Relation to Children's Altruism and Conscience Development. In Small Groups: Social Psychological Processes, Social Action and Living Together, Hare, A. P., Blumberg, H. H., Kent, V., Davies, M. (Eds.), John Wiley and Sons, London.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02138-03 LDP

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Undernutrition and Behavioral Competence in School-Age Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian Radke-Yarrow	Chief	LDP	NIMH
Other:	David E. Barrett	Research Psychologist	LDP	NIMH

COOPERATING UNITS (if any)

Department of Neurosciences, School of Medicine, University of California at San Diego, LaJolla, California 92037

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

Total Staffyears:	PROFESSIONAL:	OTHER:
.40	.10	.30

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the present study the effects of nutritional deficit at different periods in early life on the child's behavioral competence at school age were investigated. Cognitive abilities and sensorimotor skills were also assessed. Sixty-five 7-year olds from very low economic Anglo families in Southern California were the subjects. Information about nutritional histories and current health was obtained from a mother interview, anthropometry, and hemoglobin analyses. Children were observed in 6-person groups for a total of 5 hours. Natural and experimental situations were involved. Individual cognitive and sensorimotor tests were administered. Nutritional risk during pregnancy was significantly related to several measures of social interaction and affect: low social responsiveness, diminished affect, and dependency. In contrast to the results for social-emotional measures, cognitive and sensorimotor variables were not predicted by prenatal nutritional risk.

PHS-6040
(Rev. 2-81)

Project Description:

Little research has been directed to the effects of undernutrition on children's behavioral competence. However, research on malnourished animals identifies a critical complex of behavioral abnormalities. Animals with a history of undernutrition are described as showing apathy, passivity, inability to sustain attention, reduced exploration and curiosity, fearful avoidance of new stimuli, failure to respond normally to social initiations from other animals. In the present study, the effects of nutritional deficit at different periods in early life on the child's social-emotional behavior at school age were investigated. Cognitive abilities and sensorimotor skills were also assessed.

The children in this study are 65 seven-year-olds, from Anglo families, living in poor urban areas of Southern California. All families are below a poverty income level.

Information about nutritional histories and current health was obtained from a mother interview, anthropometry, and hemoglobin analyses. Children were assigned scores on five composite variables, each representing risk for developmental impairment due to nutritional stress at a different phase of development. The composites were: conditions of pregnancy, neonate health, child health from birth to two years, child health from two years to present age, and current anthropometry and hemoglobin. To control for SES factors in the data analyses, data on conditions of housing, parents' occupation and mother's education were obtained.

Children were observed in 6-person play groups for a total of 5 hours. Natural and experimental situations were involved. Six general classes of behavior were coded: behavior with peers, behavior with adults, interaction with the environment, behavior alone, activity level, and emotional expression. Individual cognitive and sensorimotor tests were administered.

The data indicate that early undernutrition has a deleterious effect on children's later social-emotional development. Prenatally undernourished children were characterized by low social responsiveness, diminished affect, and dependent behavior. It is suggested that early nutritional stress compromises the child's ability to respond to stimulation and engage the environment in interaction, resulting in early interactional failures which impede the development of later social competencies. In contrast, cognitive outcomes at school-age were not predicted by prenatal or early postnatal nutritional deficit. The findings indicate, however, that cognitive performance is strongly influenced by recent health and nutrition--factors which, it is assumed, affect the child's motivation and ability to perform in the standard test situation.

Further analyses are underway.

Addendum:

This project was carried out off-site. The data collection was performed on a contract that terminated September 30, 1979. This included case finding, screening, nutritional history and status, and the psychological measurements. The data analysis, writing, and editing of the paper has been performed under a Professional Services Contract which covers the period October 1, 1981 to September 30, 1981.

Publications:

Barrett, D. E., Yarrow, M. R., Ziegler, M., & Livingston, R. B. Effects of early malnutrition on children's social-emotional functioning at school age. In Links in the Chain: Closing the Gap (Proceedings of the Annual Conference of the Association for the Care of Children's Health), Toronto, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02139-03 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Children's Behavior in Situations of Emotional Distress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
Other:	Marian Radke-Yarrow	Chief	LDP	NIMH
	Michael Chapman	Staff Fellow	LDP	NIMH
	Ronald Iannotti	Intergovernmental Personnel Act Fellow	LDP	NIMH

COOPERATING UNITS (if any)

Laboratory of Developmental Psychology

LAB/BRANCH

SECTION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

INSTITUTE AND LOCATION

TOTAL	Staffyears:	PROFESSIONAL:	OTHER:
	.71	.27	.44

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Children's cognitive, affective and behavioral capabilities in coping with the emotional distresses of other persons are investigated. Relationships among these domains and possible changes in these relationships with age are assessed. Arousal of affect and prosocial interventions were measured in the context of several simulations of emotion. Traditional measures of perspective taking and role taking, and new tests of reasoning were administered. Twenty-two, five- to seven-year-old children who constitute a longitudinal sample were studied as were 60, four- to eleven-year-old children in a cross-sectional research design. The measures of reasoning indicate that many children, some very young, use concepts of empathy, guilt, aggression, and egocentrism to interpret other's psychological states. Children are often able to articulate complex theories to explain emotional and antisocial behaviors, and further are aware of some of the ambiguities and multiple motives underlying behavior.

PHS-6040
(Rev. 2-81)

Project Description:

Both inferential and affective processes are hypothesized as mediators of empathic capabilities. Children's abilities to comprehend another person's circumstances, i.e., role-taking or perspective-taking skills, are forms of inferential abilities that may facilitate empathic capabilities. So, too, affective arousal may lead to altruism. One purpose of this research is to examine patterns of interdependencies among children's behaviors, emotions, and cognitions. A second issue concerns developmental transitions in these three domains. The transmission of parental communication patterns is also addressed by examining the the correspondence between mothers' and children's interpretations of different prosocial moral actions.

Two samples of children have been studied in a laboratory investigation: A cross-sectional sample of five girls and five boys at each of the following ages (4, 5, 6, 7, 10 and 12 years) and a longitudinal sample of 22 girls and boys, ages five to seven, for whom there are records of the natural history of their empathic capabilities. Arousal of affect and empathic interventions were assessed in the context of several simulations of emotion. Measures of inferential ability were the Peabody Picture Vocabulary Test, forward and backward digit span, an emotion identification test, and perspective-taking tests (Flavell, Chandler). New tests of reasoning about emotions were constructed. Children's interpretations were obtained for eight story distress incidents, illustrating distresses predetermined to be within the realm of the children's own experiences. Mothers were also given these reasoning tests.

Coding has been completed. Preliminary analyses indicate that both quantitative and qualitatives assessments of children's inferential skills are very differentially estimated as a function of the different procedures. The newly constructed reasoning instrument yields many categories of children's responsiveness to distress that are not tapped by other test procedures. Prosocial and antisocial responses in the distress stories are explained by children in terms of constructs of empathy, guilt, and moralization, egocentrism, aggression, and denial. Children sometimes develop elaborate, complex theories to explain peoples' actions toward victims in distress. Many children are well able to articulate some of the ambiguities and multiple motives inherent in both acts of empathy and of nonintervention.

Publications:

Yarrow, M. R., Waxler, C. Z. Roots, motives and patterning in children's social behavior. In J. Reykowski, J. Karylowski, D. Bar-Tal, & E. Staub. (Eds.), The development and maintenance of prosocial behavior: International perspectives. New York: Plenum Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02140-03 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Interrelations of Early Cognitive Development and Empathic Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael Chapman	Staff Fellow	LDP NIMH
OTHER:	Carolyn Z. Waxler	Research Psychologist	LDP NIMH
	Marian R. Yarrow	Chief	LDP NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
.21	.17	.04

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The research objective is to examine the development of empathy and prosocial behavior in relation to progressive differentiation of self and other in the second year of life. Data on children's empathic capabilities are taken from mothers' systematic observations of their children's responses to naturally occurring emotional incidents in the home environment. Understanding of self and others is assessed by a Self Recognition Scale (children's reactions to their own mirror image) and an Agent Use Scale (understanding of other persons as independent agents in the context of pretend play). An Object Permanence Scale is also administered as a standard measure of cognitive development in the sensorimotor period. Twenty children are studied from 10 months of age until two years. Data collection is complete and analysis is under way.

PHS-6040
(Rev. 10-76)

Project Description:

Empathy, the capacity to share another person's feelings or emotions, is thought to be the primary motivation for prosocial behaviors such as altruism, helping, and cooperation. It has been further suggested that the development of empathy is related to the progressive differentiation of self and other in the second year of life. Clinical evidence suggests that disturbance in differentiation of self and other may result in later pathology.

In the present study, the development of empathy and prosocial behavior is examined in relation to progressive differentiation of self and other in the second year of life. Data on children's empathic capabilities are taken from mothers' systematic observations of their children's responses to naturally occurring emotional incidents in the home environment. Understanding of self and other is assessed by a Self Recognition Scale (children's reactions to their own mirror image) and an Agent Use Scale (understanding of other persons as independent agents in the context of pretend play). An Object Permanence Scale is also administered as a standard measure of cognitive development in the sensorimotor period. Twenty children are studied from 10 months of age until two years.

Data collection is been completed and analysis is under way.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02141-03 LDP

PERIOD COVERED
October 1, 1980 - September 30, 1981

TITLE OF PROJECT (80 characters or less)

Assessing the Progress of Children in Residential and Day Treatment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Thomas M. Achenbach Research Psychologist LDP NIMH

COOPERATING UNITS (if any)
Hillcrest Children's Center, Washington, D.C.

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, MD 20205

Total Staffyears:	PROFESSIONAL:	OTHER:
.00	.00	.00

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the study are (1) to determine the relations among ratings-made by parents, teachers, child care workers, and the independent observer--of the behavioral problems and competencies of children in residential and day psychiatric treatment; (2) to determine the relations between children's initial behavior patterns as reported by these sources and the children's progress in treatment. The initial ratings were made by parents, Hillcrest staff, and the independent observer at the beginning of the academic year and again in the late spring. Relations between initial behavior patterns--derived from ratings by the various sources and children's progress--as measured by the follow-up ratings and indices and academic functioning--are being compared with similar data on out-patient samples to determine the prognostic validity and generality of the initial behavior patterns.

PHS-6040
(Rev. 10-76)

Project Description:

Because judgments of children's behavioral problems and competencies are inevitably influenced by the conditions under which the children are observed and by the observers' relationships to the children, it is essential to identify areas of agreement and disagreement among observers and to determine the best sources of observations for assessing psychopathology, making treatment decisions, and evaluating change in disturbed children.

The purpose of this project is to extend to children in day and residential treatment the methods of assessment we have developed and applied to children treated on an outpatient basis (Project No. Z01 MH 00259). The subjects were 40 children aged 6 to 13 in treatment at Hillcrest Children's Center in Washington, D. C. The rating instruments were versions of the Child Behavior Checklist (Project No. Z01 MH 00244) that have been developed for raters having different relationships to the children (parents, child care workers, teachers, direct observers).

The design of the study entailed obtaining behavioral ratings from the following observers: Ratings on the Child Behavior Checklist from the child's parents if the child lived at home or from child care workers if the child lived at the treatment center; ratings on the Teacher Report Form from the child's teacher and teacher's aide; and direct observational ratings from a trained observer who observed the child for six 10-minute periods in class and six 10-minute periods in recess. During each 10-minute time sample, the observer wrote a narrative description of the child's behavior. From this narrative, she then rated the child on behavior problem items drawn from the Child Behavior Checklist, using 4-step response categories. She also scored on-task behavior at 1-minute intervals within each 10-minute sample.

The ratings were made in the autumn and again six months later in order (a) to determine agreement among the various observers at the beginning of a school year and again at the end of the school year, and (b) to determine which source or combination of sources of ratings best predicts outcomes over the course of the school year. In addition to behavioral ratings, achievement and cognitive measures will be used as indices of improvement.

It is expected that comparison of changes for children differing in initial behavioral patterns will provide a basis for identifying those who do and those who do not benefit from treatment as currently rendered. Comparisons are also being made with findings for outpatients in order to determine whether the predictive power of initial behavior patterns is similar for inpatients and outpatients. Data collection has been completed and the analyses are now under way.

This project has been terminated because the PI is no longer at NIMH. This is a final report.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02142-03 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Behavioral Studies of Children with Juvenile Diabetes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard A. Moss	Research Psychologist	LDP NIMH
OTHER:	Blanche S. Jacobs	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

Local Community Physicians in Private Practice

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
.98	.37	.61

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this research is to identify attitudes, concerns and response patterns that children and their parents exhibit in relation to the child's having juvenile onset diabetes for a period of years. The main focus of the research is on identifying stabilized behavioral or adaptive patterns that have evolved as a psycho-social reaction to the disease and to ascertain the factors that have led to a particular style of adaptation. Particular attention is given to whether a child develops a passive-helpless orientation or attempts to master and/or excel in some area of endeavor as a life style in coping with a chronic illness. The research involves the study of children between 11 and 18 years of age who have had juvenile onset diabetes for three years or longer. A matched control group of healthy children is also seen. Data collection consists of interviews, questionnaires, and standardized tests administered to the children and their mothers and the procurement of life event data from school and medical records. This research is considered a prototype for how children in general cope with stress.

PHS-6040
(Rev. 10-76)

Project Description

Chronic and life threatening illness in childhood presents special challenges, conflicts, and stresses for the stricken child as well as for his/her family. The purpose of the research is to identify attitudes, concerns, and response patterns that children and their parents develop as a function of the child's being ill with juvenile onset diabetes for a period of years. A major focus of this research is on coping patterns and the acquisition and use of skills in dealing with a stressful life event. A variety of factors assumed to influence the child's stabilized modes of dealing with the illness are studied. Factors include severity of the initial episode, age of the child, family behavior patterns, support systems, health attitudes, personality attributes, and parental attitudes and behaviors. The research is considered a prototype for studying how children in general cope with stress. It is assumed that juvenile diabetes involves special and standard life stress situations that dramatize developmental processes and coping patterns that are characteristic of all children.

A central hypothesis is that the behavior of children who are handicapped by some chronic adversity will be polarized toward exhibiting extremes on the dimension of helplessness or heightened mastery behavior. Children who are living with a personal misfortune will tend to be bimodally distributed whereas healthy or unaffected children will be normally distributed on this dimension.

The research involves the study of 40 children between 11 and 18 years of age who have had juvenile onset diabetes for three or more years, by which time stabilized behavioral reactions to the illness should be manifest. A matched control group of children with no history of chronic illness or permanent handicaps also will be studied. These children and their mothers are seen at NIMH for a session lasting from 2-1/2 to 3 hours. This session consists of an interview with the child, a separate interview with the mother, and the administration of a series of standardized psychological tests aimed at identifying personality characteristics, child rearing practices, goal setting behavior, and standards of competence. The interview with the child and the mother focuses on the history, affective reaction, and adaptation to the illness and with the fostering and acquisition of coping styles, standards, and evaluating helplessness and mastery orientations in the child. For the control group, only the interview and the psychological tests will be administered.

The battery of procedures administered in addition to the interview includes: (1) a level of aspiration task; (2) an anagram task; and (3) a categorical fluency task. Two Q-Sort procedures are also administered to both the child and mother in which they sort items which describe the mother's child rearing practices and psychological attributes of the child. The mothers also are administered the Jackson Personality Inventory.

This research is in progress and 18 cases have been studied. It is anticipated that about 6 months will be required to complete the data collection.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02143-03 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Psychosocial and Biomedical Interactions in Juvenile Diabetes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Beatrix A. Hamburg	Guest Worker	Children's Hospital Med. Center, Boston
OTHER:	Gale E. Inoff	Psychologist	LDP NIMH
	Michael H. Ebert	Chief, Sect. on Exp. Ther.	LCS NIMH
	Irwin J. Kopin	Chief, Lab. of Clinical Science	LCS NIMH
	Phillip Gorden	Chief, Clinical & Cellular Biology Section	DB NIAMDD
	David Newsome	Chief, Retinal & Ocular Conn. Tissue Diseases Section	CB NEI

COOPERATING UNITS (if any)
Laboratory of Clinical Science, NIMH
Diabetes Branch, NIAMDD
Clinical Branch, NEI

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
1.86	.02	1.84

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Stress influences all aspects of health status and behavioral adaptation. However, although emotional stress and psychosocial factors have all been thought to be influential in the course or onset of insulin-dependent diabetes and anorexia nervosa, there have been no systematic studies of the syndromes in terms of a psychosocial-biological, stress interaction model. This project investigates psychosocial and biomedical interactions influencing glucose metabolism in response to stress in these two illnesses. Normal volunteers will be compared with subjects who have diagnoses of either anorexia nervosa or insulin-dependent diabetes. Attempts will be made to delineate subsets of patients for whom the profiles of interactive effects of biomedical and psychosocial variables are distinctive and to correlate health consequences with patterns of response to three specific types of stress--physical stress (exercise), emotional but non-interpersonal stress, and interpersonal stress. Data collection has begun. An adjunct project investigates relationships between degree of metabolic control in diabetic children and aspects of psychological functioning expected to influence and be influenced by health status. Data analysis is currently underway.

Project Description

Study I.

The purpose of this study is to investigate psychosocial and biomedical interactions influencing glucose metabolism in insulin-dependent diabetes and anorexia nervosa. Normal volunteers will be compared with subjects who have diagnoses of either anorexia nervosa or insulin-dependent diabetes. Attempts will be made to delineate subsets of patients for whom the profiles of interactive effects of biomedical and psychosocial variables are distinctive and to correlate health consequences with patterns of response to three specific types of stress--physical stress (exercise), emotional but non-interpersonal stress, and interpersonal stress.

In considering the full impact of diabetes or anorexia nervosa, the burden of chronic severe psychosocial stress for patient and family must be recognized. In diabetes, the stress takes the form of living with the foreboding of a shortened life span for the patient, the possibility of severe complications, apprehension over the unpredictable occurrence of insulin reactions or life-threatening medical crises related to ketoacidosis, and concern over being able to competently handle these crises. At best, there are the daily problems of preemptive demands for a personal and family life style tightly structured around monitoring and regulating the diet, exercise, and medication of the diabetic member. Anorexia nervosa is a chronic, debilitating physical as well as psychological disorder in which the severe self-imposed starvation can even lead to death. Although emotional stress, psychosocial factors, and family interaction pattern have all been thought to be influential in the course or onset of these two illnesses, there have been no systematic studies of the syndromes in terms of a psychosocial-biological, stress interaction model. Recent advances in biomedical and behavioral concepts, technology, and measurement techniques now make such studies possible.

For many years diabetes was viewed as a simple hormone deficiency syndrome in which the disturbances of glucose metabolism could be reversed by exogenous insulin administration. Recent advances in diabetes research have established the multifactorial control of glucose regulation. The most prominent of the regulators that are known to increase blood sugar are also significantly responsive to stress. These are: growth hormone, glucagon, catecholamines, and cortisol. Recent studies of neuropeptides have shown that somatostatin can be a powerful inhibitor of both growth hormone and glucagon. The role of other neuropeptides in glucose metabolism is less clear. Eigler, Sacca and Sherwin have shown that physiologic increments of glucagon, epinephrine and cortisol have synergistic actions. This work suggests that stress hyperglycemia cannot be ascribed to a single hormone but is a consequence of the combined elevations of several hormones. Stress responses will be studied in both metabolically labile and non-labile diabetics and in anorexia nervosa patients.

Patients will be between ages 10-35 and will be studied as in-patients at the NIH Clinical Center. Major research issues to be studied are described below:

1. It is hypothesized that there are significant individual differences in

the patients' biomedical responses to stress. It is hypothesized that these individual differences in response to stress will systematically relate to health status. Biomedical responses to three types of stress will be assessed: (1) physical stress (exercise); (2) emotional, non-interpersonal stress (a modified version of the Stroop Color-Word Interference Test); (3) emotional, interpersonal stress (a conflict resolution task involving the family).

2. Some clinical approaches to the treatment of these patients involve the assumption that their health status is related to interpersonal stress reactions stemming from specific intra-familial conflicts and particular disturbed patterns of family functioning. The present study will examine this model by assessing biomedical responses to interpersonal stress during the experimental procedure involving the family. Systematic psychosocial assessments will also be made during this family interaction procedure. Additional characterization of family dynamics will be obtained from interview data. Biomedical responses to emotional, non-interpersonal stress and to the stress of exercise also will be examined. It is hypothesized that aberrant biomedical patterns of response to stress will be found and that these patterns of response may be triggered by emotional, non-interpersonal stresses as well as by those of an interpersonal nature. The pattern of biomedical response to exercise stress also will be examined for evidence of aberrant response.

3. Other variables to be studied include: coping patterns, locus of control, effects of degree of knowledge regarding the disorder, magnitude of denial, anxiety and depression, and psychosocial functioning of patient and family.

Study II.

An adjunct study investigates relationships between degree of metabolic control of insulin-dependent diabetes and aspects of psychological functioning expected to influence and be influenced by health status. Two hundred and twelve boys and girls, age 5-19 years, were studied during two-week sessions at a summer camp for diabetic children.

The following data were collected:

A. Psychological variables. (1) Knowledge of diabetes and diabetes care; (2) Nowicki-Strickland Locus of Control [measuring the degree to which the child feels mastery over life events and circumstances (internality) vs. the perception that factors outside the child's sphere of influence determine life events and circumstances (externality)]; (3) Behavioral problems (parental report).

B. Health status variables. (1) Control of diabetes as measured by daily, nurse-monitored tests of glucose level in the urine; (2) Number of diabetes-related hospitalizations; (3) Type of symptoms exhibited regarding insulin reactions; (4) Variables involving other health problems and health-related stresses. (5) Height and weight. (6) Presence or absence of menses.

C. Demographic variables. Age, sex, race, number of years of camp experience, number of years of having diabetes, age at onset of diabetes.

Data analyses have begun. Control of diabetes as measured by glucose levels in the urine showed different patterns for boys and girls. Degree of control increased with age for boys. For girls, there was little variability across age groups except for the decrease in degree of control for the oldest girls. (The decrease for the oldest girls appeared to reflect a relationship between poor diabetic control and continued involvement with the camp.)

For knowledge of diabetes, children's knowledge increased with age for both boys and girls; also, girls scored higher than did boys. For both sexes (but especially for boys), knowledge of diabetes was negatively related to diabetic control. The more knowledge, the worse the diabetic control. This relationship may reflect a coping effort, acquisition of knowledge, on the part of children who are in poor diabetic control.

Regarding locus of control, both boys and girls became more internal with age; also, girls were more internal control than were boys. Locus of control also was related to diabetic control: For boys, the more external, the better the diabetic control. For girls, the more internal, the better the diabetic control. (Effects of age and duration of diabetes were partialled from the above mentioned relationships with diabetic control.)

Multiple regression using knowledge of diabetes, locus of control, age, and duration of diabetes to predict level of diabetic control yielded a multiple r = .83 for males and .57 for females.

The longer one had diabetes, the more one's physical growth (e.g., height) was behind age-mates. Data analyses are nearing completion, and manuscripts for publication of findings are in preparation.

Publications:

Hamburg, B. A. Developmental Issues in School-Age Pregnancy. In Purcell, E. S. (Ed.): Psychopathology of Children and Youth: A Cross-Cultural Perspective. New York, Josiah Macy, Jr. Foundation, 1981, 299-325.

Hamburg, B. A.: Competency and Wellness in Living With Chronic Illness. In Coates, T. J., Petersen, A. C., and Perry, C. (Eds.): Adolescent Health: Crossing the Barriers. New York, Academic Press, in press.

Hamburg, B. A.: The Young Adolescent: Emotional and Social Development. In Schwarz, M. (Ed.): Promise and Performance: ACT's Guide to TV Programming for Children--Broadcasting to Young Adolescents. Cambridge, Mass., Ballinger Publ. Co., in press.

Hamburg, D. A., and Hamburg, B. A. Adaptation and Health: A Life-Span Perspective. In Kaplan, B. H. and Ibrahim, M. A. (Eds.): Family Medicine and Supportive Interventions: An Epidemiological Approach. Institute for Research in Social Science, University of North Carolina, Chapel Hill, North Carolina, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02144-02 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Child Rearing in Normal Families and in Families with Pathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian Radke-Yarrow	Chief	LDP	NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
	Michael Chapman	Staff Fellow	LDP	NIMH
	Leon Kuczynski	Foreign Visiting Fellow	LDP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

Total staff years:	PROFESSIONAL:	OTHER:
1.43	.27	1.16

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this research is the development of a research strategy (a) by which parental rearing functions and modes of behaving in the parent role are kept intact but (b) by which experimental control over selected functions or events is also exercised. A laboratory setting is used that simulates a home setting in basic respects and permits child-rearing functions and daily routines to be carried out. This research paradigm is the basis of a series of investigations. In this project a major focus is on the conceptualization and measurement of rearing. Specific aspects of rearing (affective communications and control techniques) are investigated in Projects MH 02152 and MH 02155. Rearing processes in families selected in terms of criteria of psychological disorders and of normality are investigated (MH 02156).

Project Description:

In most theories, the environment in which a child is reared is considered a major contributor to the child's development. Measurement of the familial environment is difficult, however. Conceptualization of research dimensions and variables, accessiblity of the phenomena to be studied, and techniques of assessment present enormous problems for research. The development of a research strategy by which parental rearing functions and modes of behaving in the parent role are kept intact, but by which experimental control over selected functions or events is also exercised is an objective of this research.

A laboratory setting is used that simulates a home setting in basic respects and permits childrearing functions and daily routines to be carried out. Mother and child(ren) spend three half-days in this setting, within a 2-week period. The half-day sessions are programmed to include events and interactions that involve mother and child(ren) in physical care, stimulation, control, teaching, affective interaction, and solitary self-oriented pursuits. In addition, standard interview and paper and pencil instruments relating to rearing conditions and philosophies are administered. On a fourth day, each child is seen for psychiatric assessment.

A sample size of atleast 40 families is planned. Mothers will be seen with two children, the younger child between 2 and 3 years of age and the older between 5 and 8. Supplemental data are obtained from fathers. Methodological issues in the sampling of rearing will be examined. For example, a play setting or a teaching task which is customarily used in laboratory studies as the sample of mother-child interaction will be compared with other samplings of interaction. Similarly, the results of paper and pencil instruments (mothers report on their rearing) will be compared with the behavioral measures of rearing.

This research paradigm is the basis of a series of investigations in which specific aspects of rearing (affective communications, control techniques) are studied (Projects Z01 MH 02152, Z01 MH 02155). Rearing processes in families selected on criteria of psychological disorders and normality (Z01 MH 02156).

Data collection is underway.

Publications:

Yarrow, M. R., Waxler, C. Z., and Chapman, M. Children's Prosocial Dispositions and Behavior. In P. H. Mussen (Ed.), Carmichael's Manual of Child Psychology (Vol. IV, 4th ed.). New York: John Wiley & Sons, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02145-02 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Young Children's Responses to Expressions of Anger and Affection by Others in the Family

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Mark Cummings	Staff Fellow	LDP	NIMH
Other:	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
	Marian Radke-Yarrow	Chief	LDP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff years:	PROFESSIONAL:	OTHER:
.36	.22	.14

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Developmental changes and continuities in children's responses to expressions of anger and affection by other family members are investigated. The responses of children to others' angry and affectionate interactions were studied at 1 to 2 1/2 years of age, and again at 6 to 7 years of age. Mothers were trained to observe children's responses to these incidents, monitoring children's behavior over a nine month period in the toddler portion of the study, and over a three month period in the school-aged segment. Others' angry interactions acted as environmental stresses, producing overt signs of distress from children during both age periods. However, the overall pattern of responding was more adaptive for school-aged children. Children for whom interparent hostility was frequent were adversely affected during both the toddler and school-aged periods. During both age periods, children most often responded to others' affection with attempts to join in affection and signs of pleasure. The scope of emotional development research needs to be broadened to consider the impact of emotionally laden social interactions which occur in the child's presence, but do not directly involve the child.

Project Description:

Others' emotions, even when not directed toward the child, may have an important impact on the child's affective development. This research investigates developmental changes and continuities in children's responses to expressions of anger and affection by others in the home. The responses of 22 children to others' angry and affectionate interactions were studied at 1 to 2 1/2 years of age, and again at 6 to 7 years of age. Mothers were trained to act as observers of children's responses to these incidents, monitoring children's behavior over a nine-month period in the toddler portion of the study, and over a three-month period in the school-aged segment.

Others' angry interactions seemed to act as environmental stresses during both age periods, frequently producing overt signs of distress from children. In the toddler period the probability of a distress response was heightened when others' angry interactions included physical attack. Repeated exposure to interparent anger increased the likelihood both of a negative emotional reaction by children and efforts by children to become actively involved in the conflicts. Although children were also often distressed by observing others' anger in the school-aged period, the overall pattern of responding was more adaptive. At this age, children were less likely to react with the inappropriate emotions of pleasure and anger, and were more likely to attempt to intervene empathically, when confronted with angry interactions. As in the toddler period, children for whom interparent hostility was frequent seemed to be adversely affected by these episodes. There was evidence of consistent individual differences in responding to others' anger. Children who showed little affective arousal in response to anger during the toddler period also tended not to react emotionally during the school-aged period.

There were no developmental changes in responding to others' displays of affection. During both age periods, children most often responded to others' affection with attempts to join in the affection. These affection seeking efforts were often accompanied by overt signs of pleasure. However, jealous anger responses, even among older children, were not uncommon. There were consistent individual differences across the five-year time span of the study in the tendency to respond with pleasure to observing others' affection.

Thus, by one year of age, children are detecting affective exchanges between others from the background of other interactions. They are also likely to evidence a clear emotional response to the interactions. Children develop more adequate coping skills with regard to others' anger as they get older. The findings indicate that the scope of emotional development research needs to be broadened to consider the impact of emotionally laden social interactions which occur in the child's presence, but which do not directly involve the child.

Publications:

Cummings, E. M., Zahn-Waxler, C., and Radke-Yarrow, M. Young children's responses to expressions of anger and affection by others in the family. Child Dev., in press.

Cummings, E. M. and Zahn-Waxler, C. Longitudinal trends in children's responding to others' expressions of anger and affection. Proceedings of the Society for Research in Child Dev., 1981, 69.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02146-02 LDP

PERIOD COVERED October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Etiology of Problem Aggression in Early Childhood

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Mark Cummings	Staff Fellow	LDP	NIMH
Other:	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
	Ronald Iannotti	Intergovernment Personnel Act Fellow	LDP	NIMH
	Marian Radke-Yarrow	Chief	LDP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

TOTAL	PROFESSIONAL:	OTHER:
staff years 2.23	.77	1.46

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present research is concerned with specifying the nature of early individual differences in aggression, with determining the relationship between parenting and biobehavioral factors, and the development of different patterns of aggressive behavior. Children are studied longitudinally beginning at about one year of age, and continuing for some children until seven years of age. There are two primary sources of data. The first is mothers' observations, reported in narrative, of aggressive incidents involving the child during the daily course of events in the home. The second source of data are a series of laboratory sessions in which young children's behavior towards peers and adults is examined in a variety of settings. Preliminary analyses indicate that there are considerable individual differences in styles of aggression from as early as one year of age.

PHS-6040
(Rev. 2-81)

Project Description:

Clinical research has shown that highly aggressive styles of behavior are difficult to change once they are established. There is evidence to suggest that aggressive behavior patterns may have their roots in early childhood. Such patterns of behavior may frequently be well-formed by the time a child enters school. An examination of the early development of individual styles of aggression could significantly advance both conceptual notions regarding the development of aggression and clinical approaches to the treatment of problem aggression. The present research is concerned with specifying the precise nature of early individual differences in aggression, and with determining the antecedents of the development of different patterns of aggressive behavior.

Children are studied longitudinally beginning at about one year of age, and continuing for some children until at least seven years of age. Mothers are trained to provide detailed accounts of aggressive incidents involving the child during the daily course of events in the home. In addition, children participate in a series of laboratory sessions in which their behavior towards peers and adults is examined in a variety of settings. These situations are specifically designed to elicit children's aggressive and empathic tendencies under a wide range of different circumstances; thereby allowing for a more complete deliniation of individual differences in styles of aggressive and empathic behavior. Finally, information is obtained concerning the mothers' techniques of coping with aggressive behavior by examining how she deals with the large number of different aggressive incidents she is confronted with in the home and in the laboratory. In addition, mothers participate in several structured situations that require her to demonstrate her discipline and control practices. Mothers (and in some cases, fathers) are also required to complete a series of standard instruments designed to assess intervention practices in the home.

A substantial body of data has been collected, and some facets of decoding and analysis are under way. Even at this early point in decoding and analysis, it is quite clear that there are significant individual differences in styles of aggression from as early as one year of age. In addition, preliminary analyses indicate that these early patterns tend to be consistent over time. It is notable that even as early as one year of age, the level of aggression demonstrated by some children already poses a significant problem for parents and others in their social environment.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02147-02 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Reasoning and Power Assertion in Parental Control

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Leon Kuczynski Visiting Fellow LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL staff years:	PROFESSIONAL:	OTHER:
1.13	.42	.71

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study examines parental disciplinary techniques and other methods of influencing the behavior of children. The situational determinants of these techniques such as age of children, nature of children's transgressions and nature of parents' child rearing goals as well as the effects of specific techniques on children's behavior will be investigated. In the first phase of this study, 64 mothers and their 4-year old children were studied using a laboratory compliance task that assessed mother's influence techniques and their effects on children's immediate and long-term compliance. The second phase will focus on discipline with children of different ages. Data on naturally occurring discipline will be obtained from detailed descriptions of children's behavior and parental influence strategies made by parents trained in observational recording. Data will also be obtained from recorded interactions of parents and their children in standard influence interventions in the home and in the laboratory.

PHS-6040
(Rev. 10-76)

Project Description

This study investigates the effects of specific forms of parental influence techniques on children's behavior and the situational determinants of parental choice of discipline. One aim is to study the frequency, kinds, and effects of influence techniques used by parents for children of different ages. Little is known about the developmental history of discipline. Although theory suggests that both the effects and form of discipline vary with age few studies have directly investigated the nature of parental influence as children grow older. Current conceptualizations of parental influence are also inadequate. Previous research has been hampered by a reliance on a limited number of global categories of discipline such as "reasoning" and "power assertion." Such categorizations are not unitary, for different forms of reasoning and power assertion differ in their effects. Studies that have begun to examine the role of previously unexplored forms of influence also suggest that discipline has been conceptualized too narrowly. In this study disciplinary strategies are investigated in greater detail than previously and a particular emphasis is placed on characterizing the role of reasoning and power assertion in children's compliance.

The determinants of parental choice of discipline are also investigated. Parents possess large repertoires of disciplinary and influence strategies. Recently, interest has focused on why parents choose one technique over another in dealing with particular situations. For example, when do parents primarily use force to change behavior and when do they use reasoning either alone or in combination with power assertion? One approach of this study is to investigate how the nature of children's misbehavior influences choice of technique. Specific transgressions may elicit characteristic forms of discipline. One overlapping perspective is that choice of discipline is determined by the long- or short-term nature of the behavior change that parents wish to elicit. For example, parents may use unexplained commands and power assertion to elicit children's immediate compliance while strategies such as reasoning may be used to secure a more enduring form of compliance that will persist in parents' absence.

Data on discipline are obtained using both laboratory and naturalistic methods. In the first phase of this study an experimental method was used. Mothers' attempts to secure children's compliance on a standard task and the effects of these techniques on children's compliance in mothers' presence and in their absence were studied. Parental choice of discipline was investigated with regard to two variables--the long- or short-term nature of the compliance task and the compliant vs. oppositional nature of the children themselves.

In the second phase of this project, data are obtained from 3-, 7-, and 12-year old children and their parents during naturally occurring disciplinary episodes and standard influence interventions in the home. Data on the determinants and effects of influence strategies will be obtained from detailed reports of mothers trained in observational recording (see Protocol 80-M-68 for details on method). Special attention will be paid to parents' spontaneous use of "reasoning" as well as to their reactions to selected chronic transgressions of their children.

Data for the first phase of this project has been collected and are in the stage of analysis and preparation for publication. Data collection for the second phase will be soon underway.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02148 02 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Bilirubin and Affective Dysfunction in Pre-term Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
Other:	Sarah L. Friedman	Guest Worker	LDP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL staff years:	PROFESSIONAL:	OTHER:
.14	.05	.09

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Children born premature are at risk for problems in physical, neurological and affective development. One potential biochemical precursor of dysfunction is (blood) bilirubin. Bilirubin, which causes jaundice, is neurotoxic: At high levels (>20 mg. %) it causes mental retardation. Bilirubin levels in preterm infants are often elevated. It is hypothesized that a significant portion of the diverse, adverse sequelae of preterm births may result from the presence of this neurotoxin, bilirubin, which can result in brain damage shortly after birth. The purpose of this research is to determine if the amount of impairment in early affect expression (cries), neurological performance and visual function in preterm infants is associated with concentration of bilirubin in the blood.

PHS-6040
(Rev. 10-76)

Project Description:

Preterm infants constitute a population that is at risk in several respects. They experience significant (a) mortality and morbidity, (b) physical health problems, (c) impaired inferential skills, (d) visual problems, (e) difficulties in regulation of affect. While some of the correlates of preterm births have been identified and while many of the problems associated with early birth are known, direct causal connections between specific pre- and post-natal processes and specific biological and behavioral outcomes are difficult to infer. In a significant portion of the diverse, adverse sequelae of preterm births, the neurotoxin bilirubin, which damages many regions of the brain shortly after birth might be implicated. The purpose of this research is to determine if the amount of impairment in affect expression, motor performance, and sensory performance of preterm infants can be predicted from the concentration of bilirubin in the blood.

Behavioral data were obtained on 45 preterm infants in individual testing sessions. Each child was tested at expected date of birth. The measures included visual attention as measured in terms of orientation, attention level and response decrement to a repetitive visual stimulus, (2) neurological function, as assessed by the Parmalee neurological examination, and (3) affect expression, measured in terms of (a) irritability and soothability, and (b) infant cries tape-recorded during the neurological examination. The biochemical and medical data are in hospital records and informed consents for this information were obtained this past year. All but a few of the mothers were located and consents were obtained. Neurological and visual data have already been analysed. A specialist in spectrographic and auditory analyses of cry sounds has been obtained to code the cries.

The study of preterm infant cries may begin to provide a link between some of the biological and behavioral problems faced by significant numbers of preterm infants. The preterm cry has been described as aversive, stressful, and disorganized. Some studies find preterm infants to be at risk for abuse and neglect: Their disorganized affect might elicit primitive, disorganized and aggressive behaviors in some caretakers. For other caretakers, the cry may signal that special care and support are needed. Our understanding of these interactive processes should be enhanced by increased knowledge of the origins of the affective expressions that the preterm child brings to its environment.

Publications:

Friedman, S. L., Jacobs, B. S., and Werthmann, M. W., Jr. Preterms of low medical risk: Spontaneous behaviors and soothability at expected date of birth. Infant Behavior and Development. In press.

Friedman, S. L. and Jacobs, B. S., Sex differences in neonates' behavioral responsiveness to repeated auditory stimulation. Infant Behavior and Development. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 MH 02149-02 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Mother' and Children's Reactions to Infant Cries

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
OTHER:	Sarah L. Friedman	Guest Worker	LDP NIMH
	E. Mark Cummings	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL staff years:	PROFESSIONAL:	OTHER:
.36	.17	.19

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Infant cries are complex stimuli, capable of eliciting both empathic and aggressive behaviors in others. Cries of preterm infants have been described as aversive, and as potential elicitors of child abuse. This research examines developmentally, children's response patterns to preterm and full-term infant cries. Children's affective and behavioral reactions to a crying infant were obtained. Later, children made ratings of intensities of emotions (empathy, fear, anger) aroused by tape-recordings of cries. Children ranging in age from 4 to 11 showed similar levels of empathy and similar rates of empathic interventions in response to preterm and full-term infant cries. They expressed more anger in response to full-term than to preterm cues. A majority of children were able to verbally distinguish between the two cry types along dimensions of abnormality and illness.

PHS-6040
(Rev. 10-76)

Project Description

Infants' cries provide signals to others for caretaking and social stimulation. They serve to extricate young children from situations of danger and distress. Cries are also aversive and may produce tension or anger in the listener. Thus, the cry is a complex stimulus, capable of eliciting both empathic and aggressive behaviors in others. Cries of premature infants have been described as grating and aversive. Spectral analyses, too, indicate unique characteristics of some premature infants' cries. This research departs from existing research in using both semi-naturalistic and experimental procedures to examine, developmentally, children's empathic and aggressive responses to infant cries.

Sixty middle-class boys and girls were studied at three ages: 4 to 6, 6 to 8, and 10 to 12 years. To assess empathic behavior, each child heard a tape-recorded cry in an adjacent room. Then a mother, carrying her infant, entered to look for her "crying" baby's bottle. Half the children overheard a premature cry and half heard a full-term cry. Later, each child listened to a tape-recording of a premature and a full-term infant cry. Children rated both the health of the infant and the emotions (fear, anger, empathy) aroused by the cries. The cries were then replayed and children provided verbal descriptions of the content.

Children showed similar levels of emotional arousal while overhearing premature and full-term cries, and similar frequencies of positive interventions. Certain cries resulted in more interventions than other cries, but these differences were not a function of whether the cry was that of a preterm or a full-term infant. Positive interventions increased with age irrespective of cry category. Empathy, more than fear or anger, was the emotion reported most frequently to be aroused by cries and intensity of empathy was equivalent for both categories of cries. Cries aroused anger principally in younger children, with more anger expressed toward the full-term than the premature cry. Most of the children discriminated between preterm and full-term cries, in diverse ways labeling the preterm cry as abnormal or sick.

The implications of cries of children at risk are frequently addressed within undimensional conceptual frameworks, e.g., it is commonly stated that preterm cries are aversive and 'invite' child abuse. These data on children's responses to cries, in conjunction with experimental data on adults' responses to cries (described in Z01 MH 02149-01 LDP), suggest greater complexity: In children, empathy and positive intervention responses to preterm cries. In adults, preterm cries are not consistently perceived more negatively than full-term cries.

Publications:

Friedman, S. L., Zahn-Waxler, C., and Radke-Yarrow, M. Perceptions of cries of full-term infants. Infant Behavior and Development, in press.

Zahn-Waxler, C. Children's responses to cries of premature and full-term infants. Proceedings of the Society for Research in Child Development, 1981, 302.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 02150-02 LDP

PERIOD COVERED October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Effect of Transition on Children's Social Competence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Editha D. Nottelmann Staff Fellow LDP NIMH

COOPERATING UNITS (if any)

Montgomery County Public School

LAB/BRANCH Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is focused on the relationship between children's experience with discontinuity in their lives. Specifically, children are studied in transition from elementary school to middle school and to junior high school as they enter adolescence. Children's competencies are examined longitudinally across school settings, from their own perspective as well as from that of their teachers and peers, before and after transition. The measurements include (a) children's perceptions and teachers' ratings of their social competence, (b) children's perceptions of their social self, and (c) children's friendship patterns, based on self- and peer-report. These indices of social competence are examined in relation to (a) children's perceptions and teachers' ratings of their cognitive, physical, and general competence; (b) teachers' ratings of children's over-all academic standing; (c) children's perceptions of their school environment, and and (d) indices of children's physical maturation. For a subsample of these children, competence indices also will be examined in relation to their environments outside of school.

PHS-6040
(Rev. 2-81)

Project Description:

This is a longitudinal study that is focused on the relationship between children's competencies and experience with discontinuity in their lives. Specifically, children are studied in transition from elementary school to middle school and to junior high school as they enter adolescence. Their competencies are examined across school settings, from their own perspective as well as from that of their teachers and peers, before and after transition.

The transition from primary to intermediate school has been selected for study because it represents significant culturally imposed change for a large number of children in our society and also because it coincides with the onset of puberty for many of these children. It involves change that is sufficiently far reaching to constitute a sharp discontinuity in children's lives. Many developmental and behavioral problems of children appear at this time. Although children's competencies in one setting have been found to be predictive of their competencies in similar settings, there is evidence that this predictability does not extend to new or different environments. The intersection of the demands of new environments with the developmental demands and issues of later childhood and adolescence is investigated.

In this study, we are concerned with how children feel about themselves, with their ability to make friends and find peer support, and with their general adjustment and psychological well being. Also, we are concerned with how their social competence is influenced by major change in their peer networks and environment. The research is designed to clarify how children's experience with school-peer discontinuities may change their self-perceptions and affect the way they are perceived by and interact with others. Measurements are made early in the period of transition and also later, after they have had an opportunity to adapt to their new environment.

Approximately 450 children are participating in the assessments in school. For most of these children, the relationship between discontinuity and social competence is examined in the limited context of their peer-school environment. However, a subgroup of these children also is participating in in-depth interviews about their environments outside of school, and their parents are completing questionnaires to provide similar information. Therefore, the effect of discontinuity on children's social competence also is examined in relation to the environments provided by their family and friends--or, in the context of all of the environments that constitute their world.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02151-02 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Social Cognition and Cognitive Capacity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Michael Chapman Staff Fellow LDP NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
.16	.12	.04

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The relation of children's social cognition to other cognitive abilities is investigated. In particular, social perspecitve taking as assessed by the Chandler Bystander task is compared to backward digit span performance, a commonly used measure of working memory (Case, Pascual-Leone). From an analysis of the Chandler task, it was predicted that the ability to hold and coordinate three items in working memory is prerequisite for a nonegocentric response on this task. The results supported the hypothesis: Only children who correctly reversed three or more digits on the digit span task gave non-egocentric responses on the perspective taking task.

PHS-6040
(Rev. 10-76)

Project Description:

Children's social perspective taking is investigated in relation to other types of cognitive abilities. Cognitive developmentalists have generally held that perspective taking is a function of decentration and have supported their view with evidence that children begin to give nonegocentric responses on commonly used perspective taking tasks at about the same age as they begin to solve other types of tasks (e.g., conservation) thought to involve decentration. Others have emphasized the importance of the "cognitive difficulty" of particular perspective taking tasks and have demonstrated that, if tasks are simplified in certain respects, even young children seem capable of taking another's perspective. The problem is that there has been no common agreement as to what constitutes the "cognitive difficulty" of different tasks. The present project represents an effort to evaluate the "difficulty" of perspective taking tasks in terms of the demand placed by those tasks on subjects' working memory (Case, Chapman, Pascual-Leone). From an analysis of the Chandler Bystander perspective taking task, it is predicted that only children who are capable of coordinating three independent items in working memory should be able to give a nonegocentric response on the Chandler task.

The Chandler task together with the Peabody Picture Vocabulary Test and the forward and backward digit span tasks from the Wechsler Intelligence Scale for Children were given to a sample of 40 4-, 5-, 6-, 7-, and 8-year-olds, equally divided between boys and girls. Backward digit span is a commonly used measure of working memory (Case, Pascual-Leone), while the Peabody test provides a general measure of verbal IQ. The results supported the hypothesis: Without exception, only children who were able to reverse three or more digits also gave nonegocentric responses on the Chandler perspective taking task. Verbal IQ was also correlated with perspective taking, although not as strongly as backward digit span. Backward digit span was a better predictor of perspective taking than age. No sex differences were found.

These results indicate that this approach to the study of perspective taking and of social cognition in general is worthy of further investigation. From detailed analyses of perspective taking tasks it should be possible to specify exactly what items (schemes) are involved in perspective taking performances.

This is a final report.

Publications:

Chapman, M. Pascual-Leone's theory of constructive operators: An introduction. Hum. Dev., 24: 145-155, 1981

Chapman, M. Egocentrism and Mental Capacity: A Neopiagetian Analysis. In Meacham, J. A. (Ed.), Social Development: Piagetian Perspectives. New York, Karger, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 02152-02 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Discipline and Parental Control

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leon Kuczynski	Visiting Fellow	LDP	NIMH
Other:	Marian Radke-Yarrow	Chief	LDP	NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
	Michael Chapman	Staff Fellow	LDP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL staff years:	PROFESSIONAL:	OTHER:
1.21	.27	.94

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This investigation is concerned with the measurement and conceptualization of parental control of children's behavior. This study is part of a series of investigations, the basic research paradigm for which is described in Annual Report MH 02144). Children's misbehaviors and mothers' discipline and influence techniques are observed during a series of half-day sessions in a laboratory setting that simulates a home environment. Data on maternal influence will be based on naturally occurring controls placed on children in the laboratory setting as well as experimentally elicited influence interactions. The range of control techniques used by mothers, the effects of these techniques on children's behavior and the role of children in influencing the kinds of discipline used by parents are investigated. Another focus is on the relationship between specific kinds of misbehaviors and the kinds of controls elicited by them. Finally, the acquisition of specific aspects of self-control over time and parents' reactions to reoccurrences of the same misbehaviors will be documented.

PHS-6040
(Rev. 10-76)

Project Description:

This study is concerned with issues of parental discipline and control over children's behavior. Other aspects of child rearing are investigated in Projects 02144, 02155, and 02156 of this series. Central to the present investigation is the conceptualization and measurement of parental influence. Theory and research on children's learning has been guided by inadequate conceptualizations of parental influence. Chief among these has been an excessive reliance on a unidirectional model of discipline. In this model parents are characterized in terms of their use of a limited number of disciplinary techniques and these techniques are studied only in terms of their influence on children's behavior. One problem with this approach is that discipline has been conceptualized too narrowly. Traditional categories of discipline such as "reasoning" and "power assertion" are not unitary--different forms of these techniques differ in their effects. Research is also making clear that reasoning and power assertion form only a subset of parental repertories of techniques and many potentially important forms of influence have been left unexplored. One purpose of this study is to describe in terms of more discrete categories than previously parental influence techniques and to study their effects on children's behavior.

Another aim of this study is to investigate mothers' choice of discipline in response to different categories of their children's misbehavior. Mothers do not use discipline or other influence techniques independently of their children's behavior. They probably adjust their methods according to the nature of children's transgression and to their children's specific needs for care. By studying whether specific categories of children's behaviors elicit characteristic patterns of discipline, it may be possible to move away from descriptions of parents in terms of what techniques they use to a consideration of how they use their techniques and whether specific techniques are used appropriately.

Another purpose of this investigation is to study children's acquisition of specific aspects of self-control over time and to observe parents' reactions to reoccurrences of the same misbehaviors. Most of the available data on the effects of discipline are based on discrete sequences of disciplinary interactions. Usually, the effects of single administrations of control techniques on children's immediate compliance are studied. In the natural environment, however, children are likely to commit the same transgressions repeated over time, on each occasion eliciting different controls from their parents before the behavior finally comes under control. Only a longitudinal analysis of discipline will permit researchers to go beyond the study of immediate effects of specific control techniques to a consideration of long-term influences and additive effects of discipline.

Data for this project is being collected from observations of normal and clinically depressed mothers and their children in a series of half-day sessions in a laboratory setting designed to simulate a home environment. Both naturally occurring controls placed on children by their mothers in this setting as well as experimentally elicited disciplinary interactions serve as data.

Data collection is under way.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02153-02 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Maternal Recall of Child's Early Experience

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Penelope K. Trickett	Senior Staff Fellow	LDP NIMH
OTHER:	Marian R. Yarrow	Chief	LDP NIMH
	Carolyn Z. Waxler	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
.37	.22	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study, the relation between mother's recollections of certain child behaviors and child-rearing techniques and parallel information obtained at an earlier time is investigated. Issues of maternal recall of children's early characteristics are of special relevance in etiological questions of psychopathology. Although previous research has demonstrated that in general the correspondence between these two sources of data is low, there is still much to be learned about what determines when mother's recollections are more or less accurate. (1) Are certain kinds of information recollected more accurately than others? (2) How does the mother's current view of the child color her memories? The subjects are mothers of 26-month-old children who are part of a study of etiology of behavior problems. Mothers are currently observing and reporting child behavior and parental rearing behavior. They will also reconstruct their parallel observations of the child's behavior and their own behavior when the child was 18 months old (Z01 MH 00275).

Project Description:

Much of the knowledge in the field of child development on childrearing techniques has been derived from information obtained by interviewing mothers. Some of these interviews have been frankly retrospective, asking mothers to recall certain characteristics of her child or her child-rearing techniques when her child was at a younger age. Other interviews concentrate on the present but still rely on retrospection to the extent that they require the mother to use memories of previous experiences in order to make generalizations about her own or her child's typical behavior. In this study, the relation between mother's reconstructions of certain child behaviors and child-rearing techniques and parallel information obtained at an earlier time is investigated. Issues of maternal recall of children's early characteristics are of special relevance in etiological questions of psychopathology.

Previous research (Yarrow, Campbell, & Burton, 1970) has demonstrated that the correspondence between mothers' recollections and parallel information obtained at an earlier time is often low and that systematic biases in retrospection can occur. However, there is still much to be learned about the factors that determine when mothers' recollections and generalizations are apt to be more or less accurate. For example, are certain kinds of information recollected more accurately than others? How does the mother's current relationship to the child color her memories?

Mothers in the current research are part of a clinical study of etiological factors in the development of behavior problems of children. The children are now 26 to 28 months of age. The baseline data are (a) observational records by mothers who have been trained as observers and have been providing, on a longitudinal basis (10 to 24 months), rich data on their children's responses to emotions, transgressions, and prosocial behavior and on their own discipline techniques, (Z01 MH 00275), and (b) investigators' assessments of the mother-child relationship and of child behavior.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02154-02

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Comparison of Individual Behavior Problems Before and After Treatment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jerome H. Blue	Staff Fellow	LDP NIM
Other:	Thomas M. Achenbach, Ph.D.	Research Psychologist	Univ. of Vermont School of Medicine

COOPERATING UNITS (if any)

University of Vermont School of Medicine
Burlington, Vermont

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
.56	.22	.34

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present study is designed to analyze change in individual behavior problems of children. Several hundred children from an outpatient clinic were given the Child Behavior Checklist (CBCL: Achenbach) which is a standardized instrument for assessing children's problems and competencies. Correlations were performed between behavior items reported on the CBCL at intake and follow-up to assess stability and change in behavior over the course of treatment. The findings indicate that the controversy over the relative stability or lability of individual differences in behavior cannot be answered in an all-or-none fashion for clinically important behaviors of childhood.

PHS-6040
(Rev. 10-76)

Project Description:

Many times clinical interventions are inappropriate for the particular needs of children with behavior problems. Intervention for transitory problems may unduly stigmatize a child, and lack of effective treatment for other problems may cause long-term debilitation. The research focuses on these issues of intervention by examining stability and change of individual behavior problems in children.

In addition, previous analyses of data on children with behavior problems have mainly focused on global measures of behavior. The present study is designed to make a molecular analysis of individual behavior problems using a standardized instrument for assessing child behavior problems and competencies (CBCL). The data were obtained, in previous studies, on several hundred children in an outpatient clinic, at intake and after treatment.

The design entails an analysis of change or stability in individual behavior items. The instrument is a standardized inventory of adaptive and maladaptive child behavior and consist of 118 behavior items designed to be rated by parents on a 3-point scale. Two age groups, 6-11 and 12-16, of each set were examined. The scale was completed by the parents of 468 children at the time of the children's intake and again 6 months and 18 months thereafter. Statistical analyses have been performed to determine which problems are transitory and which are resistant to change.

Some deviant behaviors show great stability with respect to both rank ordering and absolute level. Other behaviors show major changes in level, but the changes in level are so uniform within a cohort that individual children retain stable rank orders over time. Still other behaviors remain at a constant level within a cohort, but individual children change their rank ordering relative to peers. Some behaviors show considerable change whether viewed in terms of children's rank order or the level prevailing within a cohort. The findings indicate that the controversy over the relative stability or lability of individual differences in behavior can not be answered in an all-or-none fashion for clinically important behaviors of childhood. In fact there are a number of stable and transient behavior problems with differing implications for treatment. The analyses are continuing.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 MH 02155-02 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Affective Communications between Depressed Mothers and their Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
Other:	Marian Radke-Yarrow	Chief	LDP	NIMH
	Michael Chapman	Staff Fellow	LDP	NIMH
	Leon Kuczynski	Visiting Fellow	LDP	NIMH
	Leon Cytryn	Research Psychiatrist	BPB	NIMH
	Donald McKnew	Research Psychiatrist	BPB	NIMH
	Linda Stern	Student Scientist	LDP	NIMH
	Janice Krupnick	Guest Worker	LDP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staffyears:	PROFESSIONAL:	OTHER:
1.96	.47	1.49

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a study of the affective environments and the child-rearing practices that depressed and nondepressed mothers provide for their young children. Parent-child interaction and emotional development in children are assessed. Several questions are addressed: (1) Do young children of depressed mothers more frequently show symptoms of general disturbance and/or symptoms of depression than children of nondepressed mothers? (2) Are there differences in these children's adaptive and maladaptive behavior patterns (aggression, empathy, guilt, self-esteem)? (3) Do different kinds of parental depression (unipolar, bipolar, neurotic) differentially affect children's behavioral competence? Mothers and fathers are screened for psychopathology with the SADS. Mothers with two siblings (2-3 years and 5-8 years) are studied in laboratory sessions in which assessments of parent-child and child-child interaction are made. also, children and mothers are seen individually for assessments of child psychopathology and parental perceptions of child-rearing practices, respectively.

PHS-6040
(Rev. 10-76)

Project Description

Most conceptualizations of transmission of psychopathology, whether based on biological processes, learning principles or psychoanalytic constructs, would predict that parental depression would influence children's affective development. Many mechanisms of transmission are possible. In addition to genetic and biochemical factors, a number of different environmental influences may be present: atypical discipline and childrearing practices; observational learning (i.e., the child's continuous viewing and subsequent adoption of behaviors of depressed adult models); the parental conditioning of "learned helplessness;" contagion of affect (the child "feels" the mood of the depressed parent) and so on. A first step in this area of research, involves examinations of the environments in which children of depressed parents are reared, and the behavioral outcomes for children.

Several questions are addressed: (1) Do young children of depressed mothers more frequently show symptoms of general disturbance and/or symptoms of depression than children of nondepressed mothers? (2) Are there differences in these children in terms of specific domains of adaptive and maladaptive functioning (e.g., empathy, aggression, guilt, self-esteem, social withdrawal)? (3) In terms of those child variables just described, what are the effects on children of different kinds of parental depression? (4) Do mothers exhibit their psychopathology differentially with different siblings such that some children in families with parental illness may be at greater risk than other children? (5) Can the generalized impact of the mother's rearing be discerned by observing the interactions between the siblings and the quality of care provided for the younger sibling by the older sibling?

Mothers are diagnosed with the SADS (screening for schizophrenia and affective disorders) and women are selected for one of three different experimental groups depending upon whether they have bipolar, unipolar, or neurotic depression. Control groups of nondepressed mothers are studied. Fathers are also screened. The families are middle- and lower-class and have two siblings in the appropriate age categories for study, one between the ages of two and three and one between the ages of five and eight. After an initial home visit, the mother and younger child come to NIMH for three laboratory sessions in which multiple measures of parental rearing practices and child functioning are obtained (described in Annual Report # 02144). The older child also participates in the third session. In session four, each child is diagnosed by a psychiatrist: The Child Assessment Schedule (CAS) is used for the older child, and a structured prospective assessment situation is used with the younger child. The older child then participates in experimental procedures in which assessments of guilt, empathy, aggression, and self-esteem are made. Children's abilities to establish relationships outside the family setting are also assessed. The mother provides information on her perceptions of (a) her child-rearing practices (Block Q Sort), (b) the familial affective environment, and (c) the presence of symptoms of problem behaviors in the older child (Achenbach Check List). Fathers, too, teach and discipline

their children and may play a role in the development of children's symptomatology. Further, the kind of parental role fathers assume may become especially important when the mother is ill. Therefore, fathers perceptions of their child-rearing activities and their assumption of caregiver responsibilities are obtained.

Data collection is underway.

Publications

none

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02156-02 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Child Rearing: Effects of Maternal Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael Chapman	Staff Fellow	LDP NIMH
	Marian Radke-Yarrow	Chief	LDP NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
	Leon Kuczynski	Visiting Fellow	LDP NIMH
	Leon Cytryn	Research Psychiatrist	LDP & BPB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
1.16	.32	.84

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Child rearing behaviors of parents with psychopathology (depression) and the effects of these behaviors on children's development are studied. Children of depressed parents are known to be at risk for the development of psychopathology. This study will attempt to determine through what mechanisms parental pathology manifests itself in child rearing and affects children's behavior. It is predicted that affective communication and cognitive structuring are particularly important aspects of parent-child relations likely to be affected by parental pathology. Parents and children participate in half-day sessions in a home-like setting in the Laboratory of Developmental Psychology and their behavior is observed in a series of standard situations meant to elicit typical child-rearing interactions. This study is part of a series of investigations, the basic paradigm for which is described in Annual Report MH 02144.

PHS-6040
(Rev. 10-76)

Project Description:

Child rearing behaviors of parents with psychopathology (depression) and the effects of these behaviors on children's development are studied. Children of depressed parents are known to be at risk for the development of psychopathology. This study will attempt to determine through what mechanisms parental pathology manifests itself in child rearing and affects children's behavior. It is predicted that affective communication and cognitive structuring are particularly important aspects of parent-child relations likely to be affected by parental psychopathology. In the present study, a home-like environment is recreated in the laboratory and mothers are asked to participate in standard situations meant to recreate typical child-rearing interactions. For example, mothers will be asked to prepare lunch for the child, the child's reaction to the mother's absence and the presence of a stranger will be observed, mothers will be asked to teach children to perform age-appropriate tasks, and children's reactions to the experience of a physical examination will be observed. The present study will focus on the analysis of mothers' and children's spontaneous behaviors in these and other standard situations.

Mothers will be given an initial screening. Based on this, groups of normal and depressed mothers with 2 to 3 year old children will be selected (See project Z01 MH 02155 for criteria). From previous experience with depressed parents, it is expected that depressed mothers will exhibit less affect communication with their children, less sensitivity to children's affect expressions, and less social stimulation overall. In regard to the cognitive structure (explanation of objects, persons, and events) provided by mothers for their children, it is expected that depressed mothers will provide less cognitive structure than normal mothers. It is also expected that normal and depressed mothers will differ in the amount and type of control of children's behavior shown in the laboratory setting.

While it is known that children of depressed mothers are at substantial risk for the development of psychopathology, little is known about the precise behavioral mechanisms that contribute. By observing mothers and children repeatedly, it should be possible in the present study to isolate some of the consequences of the child rearing patterns of mothers with these forms of psychopathology.

Data collection is under way.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02157-02

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Developmental Evaluation of Infants on Chloride Deficient Diet

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard A. Moss	Research Psychologist	LDP NIMH
OTHER:	Van S. Hubbard	Clinical Associate	PM NIAMDD
	Leon Kuczynski	Visiting Fellow	LDP NIMH

COOPERATING UNITS (if any)

Pediatric Metabolism Branch, NIAMDD

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
.66	.30	.36

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In July 1979 it was discovered that a group of infants who were receiving a soy formula were not behaving and developing according to normative expectations and were exhibiting a "failure to thrive" type syndrome. Analysis of this infant formula revealed that it was deficient in chloride and that many of the infants fed this formula were suffering from metabolic alkalosis and chloride and potassium deficiences. Changes to a properly balanced formula improved their condition. There is some question, however, whether or not any permanent organic damage was sustained. A preliminary study of these infants has been initiated to investigate the possibility of long-term effects from this dietary deficiency. Our research will focus on whether intellectual, behavioral, and child rearing practices were either directly and indirectly affected by the metabolic abnormalities produced from the use of this chloride deficient formula.

PHS-6040
(Rev. 10-76)

Project Description:

In July, 1979 it was discovered through an investigation conducted by the Center for Disease Control in Atlanta, Georgia, that a number of infants who were receiving a chloride deficient formula as their primary source of nourishment exhibited physical and behavioral abnormalities. These abnormalities consisted of weight loss, failure to grow, muscular weakness, delayed motor and speech development, chloride and potassium deficiences, and a condition known as metabolic alkalosis in which the pH levels of the blood are excessively alkaline. To date, 141 documented cases of infants who were fed chloride deficient formulas have exhibited at least one episode of metabolic alkalosis. Analyses of these formulas have shown their chloride levels were about 1/5 of the amount recommended for infants by the American Academy of Pediatrics.

Once the formula was corrected there was remission of the metabolic abnormalities. The purpose of the research is to determine if there are any continuing complications or long-term sequalae associated with the chloride deficiency from the use of these infants' formulas. This project is under the direction of Dr. Van Hubbard (NIH-NIAMDD) and will consist of extensive developmental studies of the physical and psychological growth of these infants as well as a complete evaluation of their biochemical, physiological, and neurological status, carried out at yearly intervals over several years. The Laboratory of Developmental Psychology is responsible for the psychological assessment of these children.

This study was recently initiated and some of the psychological procedures are still in the process of development. The children will be an average of 24 months of age at the time of the initial assessment. They will be administered an age appropriate measure of developmental status at each annual assessment: The Bayley Scales of Infant Development to 30 months of age, and the McCarthy Scale of Intelligence from 24 months to 6 years of age. Assessment of sibling IQ's will be obtained in addition to IQ scores on a matched peer group in order to have a basis for determining if the children who were on the chloride deficient diet exhibit any loss in potential developmental level. The longitudinal design also will permit study of developmental changes and progressive decrements in level of functioning.

Measurements of children's temperament as well as their compliance and responsivity to maternal directives are also included in this project. Recent studies have shown that mothers' use of control techniques are influenced by children's own behaviors. It is suspected that both mothers' controlling behaviors and children's compliance could be affected by problems of neurological development. Data are obtained from observations of mothers and children in a directed play situation. Mothers are asked to take an active role in controlling their children's behavior by directing them to attend to and engage in a predetermined variety of activities. The control and compliance data of this project will contribute to related research programs of the Laboratory which are described in Projects MH 02152 and MH 02147. At older ages temperament and behavioral problems will be studied in greater detail to determine if this early chloride deficiency directly or indirectly influenced and disrupted these modes of functioning.

The mothers are interviewed about their perception of the developmental status of the infants, their anxiety, their fears, changes in their behavior and expectations concerning the child, the effects of this experience on family planning, and their attitudes toward and trust in institutions and other aspects of society.

Publications:

Moss, H. A. Review and Integration of Risk and Prevention Issues in Infancy. Prev. Hum. Serv., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02158-02

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Impact of the Environment on the Development of the Abused Child

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Penelope K. Trickett	Senior Staff Fellow	LDP NIMH
	Elizabeth J. Susman	Senior Staff Fellow	LDP NIMH
OTHER:	Ira S. Lourie	Assistant Director	DMHSP NIMH

COOPERATING UNITS (if any)
Division of Mental Health Service Programs, NIMH
Agencies and Institutions in the Washington Metropolitan area serving abusing families

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
1.51	.87	.94

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study focuses on the emotional development of physically abused children and the relationship between this development and the environment of the child. While clinical evidence shows that abused children are at risk for a wide range of physical and emotional problems, few controlled empirical studies exist and there is no research which relates aspects of the enduring environment of the abused child to the child's development. This study will use a multi-method approach to obtain information about the physical and psychological development of the abused child. These methods include observations, parent reports about daily interaction with their child, and level of parental frustration tolerance. The emotional development of the child will be assessed in relation to affective coping, physical maturation, interpersonal problem solving, and peer relations.

PHS-6040
(Rev. 2-81)

Project Description:

This study focuses on the development of physically abused children and the relationship between this development and the environment of the child. While there is wide agreement that the childhood victims of physical abuse are at risk for maladjustment, few controlled empirical studies bearing on this issue exist.

Research is needed to clarify the frequency and intensity of the problems in the emotional development of abused children and, especially, to investigate this problem over a wider age range than has been studied to date. It is also important to study the emotional development of abused children in the context of the rearing environment because such development is undoubtedly affected not just by the sporadic episodes of physical abuse but by the more enduring child-rearing environment.

The present study is investigating aspects of the child rearing environment, specifically those processes which may lead to abusive incidents, and relates those processes to the emotional development of the child. There is evidence indicating that frequently the immediate antecedents of physical abuse of a child involve attempts at control of the child. However, the exact nature of this relationship is unclear. Two competing hypotheses (which have been suggested as perhaps distinguishing different types of abusive parents) are (1) that abusive parents may believe that harsh physical punishment is a necessary technique if one is to rear a child adequately, or (2) that abusive parents, while not valuing physical punishment any more than other parents, tend toward out-of-control anger episodes which result from child misbehavior.

Subjects are physically abused children ranging in age from 4 to 10 years and their parents. They are recruited from several local agencies serving this population. Criteria for inclusion in the sample include being two-parent families with both parents willing to participate in the study, and one of the parents being the abuser. Cooperating agencies contact families who fulfill these criteria, briefly explain the nature of the study and obtain a signed permission letter from those families who are willing to be contacted by research personnel for a more detailed description of the research. A control group of non-abusing families is being recruited by advertisement. These families are matched to the abusing families on age, race, and sex of child and educational and occupational status of the parents. The total sample will include 60 families. Approximately one-third of the families have been seen to date.

This study uses a multi-method approach to obtain information about the child-rearing environment of these families and the emotional development of the children. The families are first seen at the Laboratory of Developmental Psychology at which time the parents provide detailed information on the developmental history of the child. Parents are also administered the Rothbart-Maccoby Role-Playing Task which presents parents with tape recorded typical child misbehavior incidents. They are asked to indicate what they would do if the

incidents were to occur in their home. Parents also are asked to respond to the Block-Q-Sort of 92 items about child-rearing attitudes and values. Finally, parents fill out the Family Environment Scale, a measure of the family psychosocial environment which includes such subscales as Cohesion, Control, Organization, and Achievement Orientation.

While the parents are taking part in these procedures, the child is, first, observed in a structured situation in order to assess his/her approach to new situations and a stranger, and then administered three tests: (a) the Peabody Picture Vocabulary Test, a standardized measure of receptive vocabulary from which a verbal IQ score is derived, (b) the Preschool or School Age Interpersonal Problem Solving Task, a measure of problem solving skill and (c) Bruininks-Oseretsky test of sensory motor development

After this, each parent, separately, is asked to work with his/her child on a structured task which requires cooperation between parent and child.

This study also includes naturalistic observations in the homes of all participating families. Parent-child interaction is observed in the home to determine the frequency, duration, and sequencing of a set of predefined behaviors. Two observers visit each family at home for 2 hours on two separate occasions within two or three weeks after their laboratory visit. These visits are scheduled during the evening when the entire family is at home. Family interaction is observed as it occurs, naturally and during structured interaction tasks which the family completes. The tasks include activities such as puzzles and electronic games.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02159-01

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Information Processing and Adaptation to Research Hospitalization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elizabeth J. Susman	Senior Staff Fellow	LDP NIMH
OTHER:	Marian Radke-Yarrow	Chief	LDP NIMH
	John C. Fletcher	Special Assistant	CC Dir

COOPERATING UNITS (if any)
Office of the Director, Clinical Center

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
.44	.35	.09

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the study is to examine child, adolescent, and adult understanding and adaptation to severe illness and treatment that involves participation in human experiments. Patients' understanding of human experimentation, through the process of informed consent, provides an arena for studying reasoning and responses related to highly stressful life events. Participants will include child, adolescent, and adult in-patients and normal volunteers at the National Institutes of Health. Methods will include interviews, systematic observations, standardized psychological tests, psychophysiological measurements, and adrenal hormone measurements. Participants will be assessed during their initial admission to the Clinical Center and three and six months later.

PHS-6040
(Rev. 10-76)

Project Description

The purpose of the study is to examine child, adolescent, and adult understanding and adaptation to severe illness and treatment that involves participation in human experiments. Patient understanding of human experimentation, through the process of informed consent, provides an arena for studying information processing and behavioral responses to the highly stressful events of illness and hospitalization. In addition to the theoretical implications of information processing of disease and treatment information, findings will have implications for informed consent procedures. There is a growing awareness that even after carefully executed informed consent procedures are carried out, patients may not fully understand the implications of their participation in research. The absence of informed consent poses serious ethical problems for individual investigators and for the future of human experimentation.

In this study, three factors are investigated in relation to patient processing of illness and treatment related information:
(a) Stress related to disease and hospitalization. The stress of illness and hospitalization affects long-term psychological development and short-term processing of information to an unknown extent. Stress related to hospital admission has been documented in studies of adrenal cortical arousal and subjective measures of anxiety. Stress also interferes with processing of novel information.
(b) Age of participant. Although hospitalization may be emotionally distressing for most individuals, empirical evidence shows that children may exhibit especially high levels of depression, anxiety, and regressive symptoms during hospitalization. How children process information regarding medical treatment, which sometimes involves painful medical procedures, is virtually unknown. Participation in research may further heighten stress related to hospitalization but children do not have the right to refuse to consent to research.
(c) Informed consent procedures. The problems of failing to understand the process of human experimentation may not lie solely within research participants. The implications of informed consent as a legal procedure has tended to pressure investigators into including highly technical and lengthy descriptions of the proposed research. This practice inadvertently renders consent forms unintelligible to many participants.

The research questions are: (1) What are the levels of reasoning and behavioral responses to illness and hospitalization for children adolescents, and adults participating in medical research? (2) How do the changes in levels of reasoning and behavioral responses to painful procedures compare at time of admission and after three and six months of participation in research treatment regimens?

Participants will be male and female children, adolescents, and adult patients and a comparison group of normal volunteers at the NIH Clinical Center. Methods will include psychophysiological measurements, adrenal hormone measurements (e.g., cortisol output), standardized psychological tests (an anxiety scale and the Peabody Picture Vocabulary Test), systematic observation, and structured interviews designed to assess knowledge of specific protocols. Participants will be assessed during their initial admission to the Clinical Center and three and six months later.

Publications

Susman, E. J., Huston-Stein, A., & Friedrich-Cofer, L. K. Conceptual tempo and its relation to naturally occurring social and self-regulatory behavior in preschool children. J. of Gen. Psychol., 137: 17-20, 1980.

Susman, E. J., Hollenbeck, A. R., Strope, B. E., Nannis, E. D., Hersh, S. P., Levine, A. S., & Pizzo, P. A. A prospective naturalistic study of the impact of an intensive medical treatment on the social behavior of child and adolescent cancer patients. J. Appl. Dev. Psychol., in press.

Susman, E. J., Pizzo, P. A., & Poplack, D. G. Coping with adolescent cancer. New York: Elsevier-North Holland, in press.

Nannis, E. D., Susman, E. J., Strope, B. E., Woodruff, P. J., Hersh, S. P., Levine, A. S., & Pizzo, P. A. Correlates of control in pediatric cancer patients and their families. J of Pediatr. Psychol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02160-01

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Development of Perspective Taking and Empathy in Early Childhood

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ronald Iannotti	Intergovernmental Personnel Act Fellow	LDP	NIMH
Other:	Mark Cummings	Staff Fellow	LDP	NIMH
	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff years:	PROFESSIONAL:	OTHER:
1.56	.72	.84

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The first forms of perspective-taking and empathy, and their differential roles in mediating early empathy and antisocial behaviors are investigated in children two to three years of age. Peer interactions and child-adult interactions were videotaped on three occasions in a home-like laboratory setting. Naturalistic and experimental measures of children's perspective-taking abilities, and mother's reports of children's inferential skills were obtained. Multiple assessments were also made of children's emotional responses (empathy) and of the different types of empathy and aggressive behaviors. The interdependencies of role-taking, affect, and behavioral measures will be examined. Perspective taking and empathy are also studied in relation to parents' role-taking skills and what caretakers teach their children about understanding the thoughts and feelings of other persons. Exploration of these different antecedents and correlates of children's early manifestations of positive and negative behavior patterns may further our knowledge of the motives that underlie different forms of altruism and aggression.

Project Description:

Perspective taking, or the ability to infer the thoughts and feelings of another person, and emotional responsiveness (empathy) have been implicated in the affective development of children. Training in perspective taking has resulted in increased positive interpersonal behavior and reduced delinquent behavior in older children. The interpretation of another's motives and feelings is important in determining one's response to the other, e.g., children who exhibit a high rate of aggressive behavior are more likely to misinterpret other's motives as being hostile. Different patterns and competencies of inferential abilities and empathy may mediate different styles of aggression and altruism. For example, children who are empathic may be less likely to show hostile aggression against other persons, whereas children high in perspective taking but low in affective perspective taking may use aggression as a means of obtaining desired objects.

Recent research indicates that two-year olds are capable of empathy and aggression and that individual differences in these behaviors may be relatively stable by early childhood. If we are to understand their development and the processes which influence this development, we must examine early patterns and individual differences in these young children. The purpose of this study is to investigate four questions: (1) Which types of perspective taking exist in young children? (2) Do they use these skills in peer interactions? (3) What are the roles of empathy and perspective taking as predictors of different patterns of aggression and empathy in peer and mother-child interactions? (4) What maternal behaviors facilitate the development of empathy and perspective taking?

Twenty-four pairs of children approximately two-years old and their mothers were videotaped in 1 1/2 hour long session designed to elicit various affective and behavioral responses from the children. For example, the setting included a limited number of toys which could be shared or could produce conflict. Opportunities for emotional response were provided by adults who expressed pleasure, anger, or distress and by a short mother-child separation. These 48 children returned to the lab approximately one month later with a new friend and the session was repeated. In a third session, the original 24 pairs returned: The design of the session was altered to increase mother-child interaction and to pose various interpersonal problems to children and mothers. Naturalistic and laboratory measures were administered to assess the parent-child interaction style of the mother, the interpersonal states of others, and the child's emotional responsiveness. The video tapes are being analyzed for patterns of aggressive, empathic, and perspective taking behaviors, for methods by which mothers teach inferential skills, and for maternal demonstrations of perspective taking and empathy. Most studies of young children have focused on spatial perspective taking. In this study the measures were expanded to include affective and cognitive perspective taking. The measures of altruism and aggression are designed to include behaviors governed by different motivational processes. Particular attention is being paid to the effect of different emotional situations of others, e.g., distress, pain, anger, and pleasure, on the child's behavior and the child's affective response to these situations. Mother's rearing techniques, perspective taking abilities, and interpretation of emotional

situations are being assessed during a home visit. Exploration of these different antecedents and correlates of children's early interactions may further our knowledge of the motives that underlie different forms of empathy and aggression.

Data analysis is under way.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 MH 02161-01 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Developmental Changes in Imitation in the Natural Environment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leon Kuczynski	Visiting Fellow	LDP	NIMH
Other:	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
	Marian Radke-Yarrow	Chief	LDP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL staff years:	PROFESSIONAL:	OTHER:
.49	.27	.22

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is concerned with the development of children's imitative behavior in natural settings. Data was obtained on 24 children over a nine month period during the second and third years of life. Sources of data consisted of descriptive accounts of imitation by mothers trained in observational recording as well as mothers' reports of new forms of imitation obtained during interviews at three-week intervals. Both immediate and delayed forms of imitation were investigated. Frequently occurring categories of imitation included nonverbal expressions of emotion, and behaviors involved in parental grooming, care taking, working and discipline.

PHS-6040
(Rev. 10-76)

Project Description:

Children's imitation of the behaviors of others has been considered to be an important process in theories of behavioral development. Many aspects of personality and other complex patterns of behavior may be acquired not through direct instruction and reinforcement but by children's observation of parents, siblings, and models outside the home.

This study is concerned with the early development of imitative behavior of young children in the natural setting. Previous research on imitation has tended to use experimental paradigms in which preschool and school-age children, whose imitative repertoires are well past the acquisition stage, are studied. Further, the experimental research on imitation has focused on variables such as characteristics of the model and the situation that govern the acquisition of imitation. The extent to which findings are valid and representative is open to question because information is lacking regarding both the early acquisition and the basic repertoires of imitation that children bring to the experimental setting. Information about the different kinds of behaviors that are spontaneously imitated by children may begin to clarify the processes to which learning by observation contributes. Experimental research has also emphasized the study of immediate rather than delayed imitation. Although delayed imitation, imitation that occurs some time after the modeled behavior, is more difficult to investigate than immediate imitation, it may also provide a better index of long-term patterns of behavior acquired through the process of observing models.

The present study extends previous research by investigating the development of both immediate and delayed imitation as it occurs in natural settings. One source of data was detailed narrative accounts of children's behaviors recorded by mothers trained in observational procedures. Reliabilities of maternal reports were assessed by comparing mothers and investigators' reports of children's imitations during home visit (percent of agreement was 91%). Mothers were also interviewed every three weeks during the data collection period and were asked to report new forms of imitation that occurred since the preceding contact. Data covered nine months of the development of 24 children in the second and third years of life.

The data have been collected and preliminary analyses indicate that during the second year of life imitative repertoires are both extensive and increasingly complex. Infrequent yet salient behaviors such as characteristic expressions of emotion and idiosyncratic behaviors of models comprise a large category of immediate imitation. Complex patterns of behavior involved in the performance of household chores, grooming, and caretaking, as well as parental verbalizations during disciplinary interactions are a source of both immediate and delayed forms of imitation. Children's imitation of aspects of parental discipline implicates imitation in the development of conscience and self-control.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02162-01

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Patterns of Psychological Functioning and Psychosexual Identity in Children with Congenital Adrenal Hyperplasia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jerome H. Blue	Staff Fellow	LDP NIMH
OTHER:	Arnold Slyper, M.D.	Pediatric Endocrinologist	Children's Hospital Washington, D.C.

COOPERATING UNITS (if any)
Children's Hospital National Medical Center
Washington, D. C.

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
.31	.22	.09

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patterns of cognitive abilities, psychosexual identity, self-perceptions, and the behavioral development of children with congenital adrenal hyperplasia will be examined. The main focus of this study is to delineate the relationships among these children's patterns of cognitive abilities, psychosexual identity, how they view themselves, and how they are viewed by their parents, on the basis of standardized tests with established norms. This investigation is designed to increase our understanding of the special needs of children with congenital adrenal hyperplasia.

PHS-6040
(Rev. 2-81)

Project Description:

This study is designed to investigate the cognitive patterns, sexual identity, self-perceptions, and behavioral development of children with congenital adrenal hyperplasia. Tests which measure patterns of cognitive abilities will be used rather than a global measure of cognition. Standardized instruments, the Perceived Competence Scale for Children, new measures of masculinity and femininity, and the Child Behavior Checklist will be used to assess children's perception of their own behavioral competencies and their behavioral characteristics from the parent's perspective. The delineation of relationships among these children's patterns of cognitive abilities, how they view themselves, and how they are viewed by their parents, on the basis of standardized tests with established norms will increase our understanding of the special needs of children who are prenatally exposed to hormones and children with other hormone abnormalities.

Many reports have been made about the cognitive functioning of children with congenital adrenal hyperplasia. Some reports have shown that these patients perform better than normal and other reports fail to replicate this finding or find the converse. In addition, assessments of psychosexual identity in these children were not empirical or standardized. Few of the tests were age-appropriate or examined for validity or reliability. Thus differences in previous reports may be due to differences in measurement and methods of rating. This study will use a larger sample than those used in the past. It will use age-appropriate measures of cognitive abilities. It will use operationally defined measures of masculinity and femininity in order to determine the influence, if any, of hormone abnormalities on behavior.

The patterns of cognitive abilities, behavioral problems and gender identity (sex role preference and personal conception of what is sex-appropriate) of children with congenital adrenal hyperplasia using empirical tests will be examined. The tests will include the Harter Scale, Child Behavior Checklist, Personal Attributes Questionnaire, the Edelbrock Sex-typing Test, the Primary Mental Abilities Test, Object-Naming Test, Color-Naming Test, and other standardized psychological tests.

Findings will be discussed in terms of the relationships between psychosexual identity, and patterns of cognitive abilities in children with congenital adrenal hyperplasia as compared to normal control children.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH-02163-01

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Psychobiological Correlates of Behavior Problems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jerome H. Blue	Staff Fellow	LDP NIMH
OTHER:	Sarah H. Broman	Acting Chief, Mental Retardation and Learning Disorders Sec.	DNB NINCDS

COOPERATING UNITS (if any)

National Institute of Neurological and Communicative Disorders and Stroke

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
.31	.22	.09

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Children with congenital malformations are studied in relation to specific behavior problems and patterns of cognitive abilities. Data on major and minor congenital malformations, patterns of cognitive abilities and behavior problems from the Perinatal Collaborative Project will be used. This systematic analysis will serve to facilitate a discussion on the congenital malformations and patterns of congenital abilities which might be related to various behavior problems.

PHS-6040
(Rev. 10-76)

Project Description:

Some studies have shown behavior disorders to occur more frequently in children with particular malformations, while other studies have shown several factors to predict behavior disorders with mixed success. Most analyses of childhood disorders have focused on either cognitive, behavioral, or physical characteristics and not on interaction between these factors. Few diagnostic categories other than hyperactivity have been analyzed and we stress that there is a need to look at correlates of other behaviors as well. In addition, few studies have examined a large number of malformations using a substantial sample of childen with behavior disorders. Cognitive and biological correlates of various behavior problems using a large sample will be investigated in order to better understand maladjustment.

Children with congenital malformations are being studied in relation to specific behavior problems that are most critical or resistant to change, like fighting, impulsivity, shyness, and others. Because congenital malformations may interfere with the acquisition of many skills and the behavioral development of children it is important to determine whether children with such abnormalities are likely to need special attention. Thus, this project is designed (1) to document the cognitive, behavioral, and congenital malformations of children with special problems, and (2) specify the interactions among these factors in childhood disorders.

Data from the Perinatal Collaborative Study which includes neurological examinations, behavior profiles, and a variety of psychological tests will be used. The neurological examinations provide the major and minor congenital malformations of these children. The behavior profiles consist of a variety of problems which have been correlated with childhood disorder or psychopathology (i.e., Fearfulness, Emotional reactivity, Non-communication, Thumb-sucking, Nail biting, etc.). The measures of cognitive abilities include subtests of the Wechsler Intelligence Scale for Children and other psychological tests.

The research hypotheses are (1) that biological factors such as congenital malformations are associated with specific behavior problems, and (2) that congenital malformations underlie later manifestations of cognitive impairments.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02164-01 LDP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Adaptation to Adolescence: Biological and Psychological Relationships

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

CO-PI:	Elizabeth J. Susman	Senior Staff Fellow	LDP NIMH
	Editha Nottelmann	Staff Fellow	LDP NIMH
	Jerome H. Blue	Staff Fellow	LDP NIMH
OTHER:	Roger S. Johnsonbaugh	Pediatric Endocrinologist	Nat. Naval Medical Center

COOPERATING UNITS (if any)

National Naval Medical Center, Bethesda, Maryland

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years	PROFESSIONAL:	OTHER:
1.07	.85	.22

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the research is to investigate longitudinally the relationship between physical maturation and adjustment during early adolescence. The biological and role changes, in general, and the timing of these changes, in particular, may exacerbate existing emotional difficulties, create new problems that result in acting out and delinquent behavior, or even lead to psychopathology. The research focuses on two interrelated objectives: (a) the examination of the relationships between the timing of maturation, using more precise measures of pubertal status than have been used in previous research, and adjustment; and (b) the examination of the sequence of reciprocal relationships among hormonal and physical changes and psychological development. Participants will include male and female 9- to 17-year-old adolescents, their parents, and peers. Each participant will be evaluated for stage of pubertal development. Psychological assessments will include standardized psychological tests and systematic observation.

PHS-6040
(Rev. 2-81)

Project Description

The purpose of the research is to investigate longitudinally the relationship between physical maturation and adjustment during early adolescence. The biological and role changes, in general, and the timing of these changes, in particular, may exacerbate existing emotional difficulties, create new problems that result in acting out and delinquent behavior, or even lead to psychopathology. The timing of maturation derives its psychological significance, in part, from the fact that there are great interindividual differences in the onset of the physical and biological changes that signal entry into adolescence. Some adolescents complete their whole adolescent development before other children of the same chronological age have begun to mature.

Adolescents are extremely sensitive to the physical changes occurring in their own bodies and continuously compare these changes to changes in their peers for validation of their own normality. If they mature early or late, their experience of uniqueness may generate stress and result in poor self-concept, isolation, and ultimately in peer or even parental rejection. There is very little basic information available on the relationship between timing of maturation and psychological adjustment. Furthermore, precise measures of hormone levels and physical growth, needed to establish timing of maturation, have not been utilized.

Similarly, there is very little basic information available about the sequence of reciprocal relationships among hormonal and physical changes and psychological development during puberty. Changes in psychological development are thought to be influenced both by changes in hormone levels and body size and proportion and by the development of secondary sex characteristics. For example, increase in physical size is a stimulus for others to expect mature, competent, and independent behavior from the adolescent. Changes in thought processes during adolescence are assumed to be influenced by central nervous system changes related to testosterone and estrogen levels.

The research questions focus on the interrelations among hormonal and physical biological changes and changes in psychological development. Participants will be 9- to 17-year-old adolescents, their parents, and peers. At four times of measurement, four months apart, the adolescents will be evaluated, in collaboration with a pediatric endocrinologist, for stage of pubertal development. A series of psychological assessments will be carried out at the same time. They will include thought process measures (e.g., subtests of the Wechsler Intelligence Scale for Children, Primary Mental Abilities Test, Embedded Figures Test, and Color-naming and Object-naming tasks) and emotional development measures (e.g., the Offer Self-Image Questionnaire for Adolescents, the Harter Perceived Competence Scale for Children, the Child Behavior Checklist, and Personal Attributes Questionnaire). Parent-adolescent and adolescent-peer interactions will be observed during collaborative decision-making sessions and coded for conflict, affect, cooperation, and other variables related to qualitative aspects of adolescent interaction.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00478-25 LN

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neural mechanisms in object perception and memory

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Mishkin	Act. Chief, Laboratory of Neuropsychology	LN NIMH
OTHER:	J.P. Aggleton	NIMH Visiting Fellow	LN NIMH
	E.A. Murray	NIMH Extramural Fellow	LN NIMH
	J. Bachevalier	Quebec Council for Health Res. Fellowship	LN NIMH
	D.P. Friedman	NIMH Staff Fellow	LN NIMH
	B.H. Turner	Professor	Howard Univ.
	L.R. Squire	Res. Career Scientist	VA Med. Ctr., San Diego, CA
	S. Zola-Morgan	Research Psychologist	VA Med. Ctr., San Diego, CA
	W.H. Overman,Jr.	Asst. Professor	Univ. NC

COOPERATING UNITS (if any)

Howard University, VA Medical Center, San Diego, CA, University of North Carolina

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

Section on Cerebral Mechanisms

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.5	2.5	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The striate cortex is the source of two major, corticocortical, multisynaptic visual pathways. One of these follows the course of the superior longitudinal fasciculus, interconnecting the striate, prestriate, posterior parietal, and dorsal prefrontal areas, and appears to be important for spatial vision. The other follows the course of the inferior longitudinal fasciculus, interconnects the striate, prestriate, inferior temporal, and ventral limbic areas, and is critical instead for object vision. Our research concerns both pathways, but this report focuses on the second, or ventral, one. Our studies suggest that each of the extrastriate links in this ventral pathway adds a different capability to object vision, making possible, in turn, highly efficient object perception, object memory, and object-affect associations. The sequential dependency of these behavioral functions is paralleled by the sequential dependency of the successive neural structures in the chain. The somatosensory cortex may also project through a ventrally directed pathway to temporal limbic areas, and parts of this system may perform functions in tactual perception, memory, and affect analogous to those described above for the visual system.

PROJECT DESCRIPTION:

The objective of the sub-projects described here is to define the role in object perception and memory of neural structures outside the primary sensory projection areas, using behavioral and lesion analyses. (1) Previous ablation studies had demonstrated that stimulus equivalence between the visual half-fields depends on the convergence of their projections onto single inferior temporal neurons. Follow-up studies using a fixation-monitoring device will test whether inferior temporal neurons account for stimulus equivalence across any retinal translation, and also whether, as suspected, it is the neurons in the posterior part of inferior temporal cortex that are mainly responsible for this stimulus-equivalence mechanism. (2) Monkeys with various cerebral lesions were compared on tests designed to measure one-trial visual memory for objects. Only removals within the anterior part of the inferior temporal cortex (area TE) impaired this object recognition ability, suggesting that this is the locus in which the visual trace left by previous viewing is preferentially established. By contrast, on tests designed to measure one-trial memory of object-reward associations, equivalent impairments were obtained both after area TE lesions and after amygdalectomy. The results thus point to object recognition and object-reward association as consecutive processes that depend in part on the sequential participation of IT cortex and the amygdala. New studies are now being undertaken in an attempt to obtain more severe impairments in these two processes by increasing the size of the cortical lesions in accord with information gathered in the 2-deoxyglucose experiments (see Project on Functional Mapping of Sensory Systems), which show the full extent of all visually related areas. (3) In the process of investigating the role of temporal-lobe structures in memory we have chanced on a result that is particularly exciting because it may help to resolve a long-standing puzzle concerning the neuropathology underlying the syndrome of global anmesia in man. This syndrome, which is characterized by a profound inability to remember new experiences, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in animals by removal of the hippocampus have largely failed. What we have found in our studies, both for visual recognition memory and for object-reward association memory, is that if the hippocampus is removed in combination with the amygdala then a profound memory loss ensues, one that is even greater than that produced by lesions of inferior temporal cortex. Furthermore, the effect of the combined removal is not all-or-none, but is graded in relation to the amount of conjoint damage to the two systems. The discovery not only opens up a new possibility for resolving the discrepancy between clinical and animal findings but also provides a new insight into the neural mechanisms of memory. Additional studies have been started in an attempt to (a) identify differences in the contributions of the amygdala and hippocampus to memory, (b) explore the interactions of these two limbic structures with the diencephalon, both anatomically and behaviorally, and (c) examine the nature and substrate of the memory ability that is preserved after destruction of the limbic system. (4) The discoveries in (2) and (3) were antedated and guided by anatomical findings indicating that inferior temporal cortex projects both directly to the amygdala and indirectly to the hippocampus via the entorhinal area. To extend and refine our knowledge of these anatomical circuits, we investigated with both degeneration and axonal transport methods the inputs to the limbic structures of the temporal lobe.

Results thus far indicate that no visual area caudal to area TE projects to the amygdala, but that all sections of area TE do, and that they do so within a circumscribed part of the lateral and basolateral nuclei. In addition, other sensory systems (i.e., olfactory, gustatory, somatosensory, and auditory) project to other parts of the amygdaloid complex in such a manner as to suggest that each sensory system has a dominant influence in a given part. Finally, we have obtained new evidence indicating that the cortico-amygdaloid projection is reciprocated by an amygdalo-cortical projection and that the latter pathway appears to have an opiatergic component through which the amygdaloid complex could exert a modulating influence on the cortical processing of sensory information. These findings will help guide further behavioral studies into the possible perceptual, attentional, and mnemonic functions of the limbic system in all sensory modalities. (5) Monkeys are being trained on a tactual object recognition problem, to test the possibility that the amygdala and hippocampus participate in memory processes involving sensory systems other than the visual system, as the results of the anatomical studies outlined in (4) suggest. In addition, cortical localization studies are being performed in the somesthetic modality with the goal of identifying sequential processing systems in touch and kinesthesis analogous to those that have been identified in vision.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Vision is perhaps the best understood of the sensory modalities in relation to the functioning of the nervous system. Yet a major problem remains unsolved in vision as it does in all sensory modalities: Delineation of the events that intervene between stimulus reception at the cortex and the observed response. The demonstration that damage to the inferior convexity of the temporal lobes in monkeys produces impairment in visually mediated behavior opened up the hitherto inaccessible area of the intracerebral processes in vision, i.e., neural activity related to vision but beyond the level of the striate cortex. Unravelling these mechanisms in vision and other modalities is aiding greatly in the solution of a general problem for psychology, viz., accounting for the intervening neural processes (thought and its breakdown) in normal and abnormal behavior.

PUBLICATIONS:

Turner, B. H., Mishkin, M., and Knapp, M.: Organization of the amygdalopetal projections from modality-specific cortical association areas in the monkey. J. Comp. Neurol. 191: 515-543, 1980.

Lewis, M. E., Mishkin, M., Bragin, E., Brown, R. M., Pert, C. B., and Pert, A.: Opiate receptor gradients in monkey cerebral cortex: Correspondence with sensory processing hierarchies. Science 211: 1166-1169, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02031-05 LN

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neural mechanisms in spatial perception and memory

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Mishkin	Act. Chief, Laboratory of Neuropsychology	LN NIMH
OTHER:	L.G. Ungerleider	Senior Staff Fellow	LN NIMH
	R. K. Nakamura	Staff Fellow	LPP NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, NIMH

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

Section on Cerebral Mechanisms

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The striate and prestriate cortex project not only ventrally, to the inferior temporal area, but also dorsally, to the posterior parietal and dorsal prefrontal areas. This dorsal projection pathway appears to constitute the visual contribution to a system for spatial perception. Many modalities undoubtedly contribute to this spatial system, which can be divided into a posterior (parietal) portion serving allocentric localization, and an anterior (prefrontal) portion serving autocentric localization. The entire system is probably critical for the spatial guidance of movement, and in keeping with this, its major cortical outflow appears to be to the motor system. In addition, however, this spatial system appears to provide an essential tonic influence on the diffuse activating system, for its removal has been found to cause a profound sensory neglect.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

The first two sub-projects described below deal with the parietal and prefrontal portions of the spatial system, respectively.

(1) Animals trained on a visual landmark test, a measure of allocentric localization ability, were prepared with posterior parietal removals in one hemisphere and complete striate removals in the other, and these were then followed by a callosal transection. Results indicate that the crossed lesions alone produced severe impairment and that, once the animal recovers, the addition of a callosal transection had only a mild effect. Thus, the posterior parietal cortex, like the inferior temporal, appears to depend heavily on striate input for its visual function; but unlike the inferior temporal, the posterior parietal does not seem to receive a heavy visual input via the corpus callosum. In a second experiment, monkeys received bilateral lesions of the striate areas representing either central vision (lateral striate) or peripheral vision (medial striate). They were tested both on a pattern discrimination task, to assess residual inferior temporal function, and on the landmark task, to assess residual posterior parietal function. On the former task, only the lateral striate lesion produced a deficit, whereas on the latter task, the two lesions produced equally severe deficits. Apparently, inputs from central vision are especially important for the pattern recognition functions of inferior temporal cortex, but inputs from central and peripheral vision are equally important for the visuospatial functions of posterior parietal cortex. Thus, although interactions with striate cortex are critical for the parietal just as for the temporal areas, the striate inputs to these regions appear to be organized differently, presumably reflecting differences in the cortical processing required for spatial vs object vision.

(2) A crossed-lesion experiment like that described above has shown that the dorsal prefrontal cortex also depends on striate input for its visual function, in this case autocentric localization as measured by the spatial delayed response test. Evidence that the function is selectively spatial comes from an experiment in which animals with selective prefrontal removals were compared on a series of nonspatial visual memory tests, including delayed object alternation, delayed color matching, and delayed object matching. In every case, animals with lesions confined to the principal sulcus relearned quickly, in sharp contrast to their permanent failure to relearn spatial memory tasks. The dimensions of the spatial deficit were examined in another experiment which compared the effects of selective prefrontal lesions on up-down delayed alternation as opposed to the customary left-right delayed alternation. In every case, lesions known to impair performance on left-right tasks (e.g., principal-sulcus removals, or inferior prefrontal ablations) had just as severe an effect on the performance of the up-down task, while removals known to have little efect on left-right alternation (e.g., periarcuate lesions) also had little effect on the up-down version. Apparently, the spatial impairment after principal-sulcus lesions is not limited to left-right confusion, but encompasses the vertical dimension as well. Further support for the conception of a spatial impairment after principal-sulcus lesions was provided by an experiment on time discrimination. In this task animals were required to respond to one position

after a short delay and to the other position after a longer delay, the two delays being presented in random sequence. Animals with principal-sulcus lesions were unaffected on this task, seriously undermining the view advanced by others that their delayed reaction impairment reflects a difficulty in temporally structuring, or "parsing", the stream of sensory stimulation to which they are exposed. The results suggest instead that the lesion produces impairment only when the spatial choice that the animals are required to make depends upon the memory of a spatial cue rather than the perception of a temporal cue.

(3) In another project (Neural mechanisms in object perception and memory) evidence was reviewed showing that visual discrimination habits depend on both the cortical visual system and ventral limbic structures. But are these two systems acting together a sufficient substrate for such habits, or does the rest of the cortex, including that in the dorsal spatial system, also play a significant role? To examine this question we restricted vision to a single hemisphere (by visually deafferenting and disconnecting the other hemisphere) and then removed all the cortex from the seeing hemisphere except for the visual and limbic systems. Unexpectedly, the operated animals appeared on awakening to be completely blind, and this total blindness lasted for one to several months, abating, if at all, only very slowly and only in part. Yet, the geniculostriate pathway in these animals seemed to be intact as judged both by visual evoked potential studies and from the subsequent histological examination of the brains.

To determine whether the blindness was the result of a disconnection of the visual system in one hemisphere from the motor system in the other, animals were prepared as before but with the forebrain commissures left intact. Yet these animals too were blind, indicating that the blindness is not a disconnection effect and suggesting further that during blindness the visual signal is not processed beyond striate cortex, for otherwise the signal should have been transmitted across the preserved commissural channels (which start at the striate-prestriate border) to the motor system. The analysis implies that the territory ablated normally provides some activating input, direct or indirect, that is necessary for cortical visual processing.

In a further study, the original lesion was subdivided in an effort to localize the area supplying the activating input. It was found that a sensorimotor lesion (Brodmann's areas 1-6) failed to produce blindness in any of three monkeys. The complementary lesion, however, consisting of dorsal prefrontal, inferior parietal, insula, and superior temporal areas, did produce blindness in three monkeys for about a month. These localization results provide additional evidence that the blindness is not a visual-motor disconnection effect, and suggest instead that it is a profound form of visual neglect, since parts of the lesion that produced blindness are known to produce neglect. Accordingly, the basis of the neglect too could be impaired processing within the cortical visual system due to loss of activating input, though a less complete loss than that producing neglect. It appears from these results that the dorsal "spatial" system normally provides an activating input (relayed perhaps through the activating system) that is necessary for the visual system and perhaps other sensory systems to func-

tion properly, and this is in addition to its contribution to spatial mechanisms. For when the animals eventually do recover their use of vision, they show permanent impairment in the ability to guide their body and limbs to visual targets; that is, they then show the expected spatial disorientation.

We have found, finally, that by extension of the lesion into the limbic system animals can be rendered chronically blind. Such preparations are now being used to test the loss-of-activation hypothesis directly by the method of unit recording in the intact visual system. Thus far, striate cortex was studied in five monkeys prepared as above to produce chronic blindness, except that the optic tract was left intact in two of the animals to provide control hemispheres. The recordings were made while the monkeys were anesthetized with nitrous oxide and immobilized with pancuronium bromide. Implanted wells and head holders were used to minimize possible sources of pressure or pain.

We isolated 244 units from the hemispheres made 'blind' by the ablation, and 83 units from the 'seeing' intact hemispheres. Of the units from the blind hemispheres, 53% were moderately or highly responsive visually as contrasted with 95% of those from the seeing hemispheres. The loss of visual responsiveness was related to a consistent histological finding of inadvertent damage to optic radiations representing primarily the lower visual field, in that there was a strong positive correlation between a cell's visual responsiveness and the vertical position of its receptive field. For example, at more than 5^{o} below the horizontal meridian, only 12% of the units were moderately or highly responsive, whereas at more than 5^{o} above the horizontal meridian, 71% of the units were moderately or highly responsive.

There was no difference between the blind and seeing hemispheres either in the proportion of responsive cells with oriented receptive fields (69% and 68%, respectively) or in the proportion of oriented cells with complex-type receptive fields (71% and 63%, respectively).

We conclude that 1) although many units respond poorly or not at all in the striate cortex of the blind hemispheres, there appear to be enough moderately or highly responsive units to support vision, particularly in the upper fields; 2) moreover, visual information is not only reaching striate cortex but is being processed there as indicated by the normal proportions of cells with oriented and with complex-type receptive fields; 3) the loss of visual processing or disconnection of the efferent visual pathway responsible for the blindness must be occurring at some point beyond striate cortex.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Spatial orientation in animals is an extremely well developed, higher order, perceptual mode. It is "higher order" in the sense that it depends not on a single sensory modality but on the coordinated functioning of several of them. Our evidence points to a dual cortical localization for this suprasensory modality in the posterior parietal and dorsal prefrontal areas. Analysis of this complex, multimodal, cognitive system in monkeys may help us understand the organization of yet another complex, multimodal, cognitive

system in man, namely, the one for language. The formal similarities between the language system (for which man's left hemisphere is specialized) and the spatial system (for which man's right hemisphere is specialized) give added support to the validity of this approach.

PUBLICATIONS:

Rosenkilde, C. E., Rosvold, H. E., and Mishkin, M.: Time discrimination with positional responses after selective prefrontal lesions in monkeys. Brain Research 210: 129-144, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02032-05 LN

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neural coding of visual stimuli in the awake monkey

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2	1	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous work in this laboratory and others has shown that the inferior temporal cortex is important for the visual processing underlying pattern recognition. Also, studies have shown that single neurons in this area of cortex are responsive to visual stimuli. We are studying two aspects of inferior temporal neuronal responses: one is the role of stimulus parameters and the other is the behavioral context in which the stimulus is presented. That behavioral context is important was shown by the finding that active visual fixation can modify the response to a visual field probe stimulus. The role of stimulus parameters in the response of inferior temporal cortex neurons will be studied using conventional bar and slit stimuli, as well as stimuli constructed from sine-wave spatial frequency gratings.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives

The inferior temporal cortex in the primate has been identified as critical for visual pattern recognition. Tasks involving discrimination and recognition of patterns can only be carried out if inferior temporal cortex is intact. Furthermore, single-unit recording studies have established that inferior temporal neurons show a stimulus-locked response to visual stimuli presented in the visual fields. These previous recording studies, most of which have been carried out in paralyzed and lightly anesthetized monkeys, have shown not only that the cells respond to visual stimuli exclusively, but also, that they have large receptive fields which always include the foveal or central region of vision.

In investigating the role of inferior temporal neurons in pattern discrimination, two factors must be considered. The first is the characteristics of the stimuli to be used, recognizing that it will be especially helpful during the data-analysis stage of the investigation to have used an orderly set of stimuli. One such set is the set of sine-wave spatial frequency gratings, the advantage of which is that the patterns are relatively easy to produce and parameters are easily varied. In addition, the mathematical characteristics of this particular set are well known and easy to manipulate. Finally, this set of stimuli allows the possibility of testing the hypothesis of one group of psychophysicists that the visual system acts as a small number of spatial frequency filters and detectors and that patterns are analyzed in a manner analogous to knowing the ratios of the spatial frequency content to which the filters and detectors are tuned. However, using sine-wave spatial frequency gratings will allow the gathering of valuable data even if the hypothesis of spatial frequency channels is incorrect.

The second factor that is likely to be important to inferior temporal neurons is the behavioral paradigm in which the stimulus is presented. The awake, trained monkey therefore seems particularly suitable for such investigation.

Methods employed

A flexible and powerful on-line computer software system has been developed for the control of behavioral experiments and for gathering single-unit, event, and analog data at high resolution with a laboratory minicomputer. This technical development was carried out in collaboration with members of the Laboratory of Sensorimotor Research of the National Eye Institute. The prototype system is currently being used to record from single neurons in the anterior part of inferior temporal cortex, area TE, while the animal fixates one stimulus and neuronal responsivity is examined with other visual stimuli.

Major findings

So far we have recorded from over 200 cells distributed throughout area TE of 3 different monkeys. In our initial experiments we found inconsistent neuronal responses to large spots and squares of light presented while the monkey was fixating a smaller spot of light in order to obtain a reward. Because of this difficulty in obtaining neuronal responses, we analyzed the stimulus situation

in order to compare it to previous situations involving anesthetized monkeys in which many neurons with large visual receptive fields had been found. A major difference in the previous studies as compared to the present one was the absence of the fixation spot. In order to overcome any influence of the fixation spot, we designed a paradigm in which the spot went off for a period of time (1000 msec.) and then reappeared. During this spot-off or "blink" period, the animal's eye movements were carefully monitored by the computer, and if the animal's eye position moved more than 1 degree in any direction, the trial was discontinued and the animal had to initiate a new trial. If the animal maintained eye position, however, a stimulus was presented during the "blink" period of the fixation spot. Under this condition about 60% of the cells encountered gave a consistent visual response. By changing stimulus parameters and stimulus positions, the following conclusions were reached. In the "blink" paradigm the exact parameters of the stimulus are not critical for obtaining a response (although in these experiments only the size and shape of a spot of light was varied). The response latencies to stimulus onset were between 80 and 200 msec. The response amplitudes were consistent but relatively small compared to those of visual-system neurons closer to the sensory input, such as striate neurons. When the same stimulus that evoked a response during the "blink" was presented while the fixation spot remained on, the evoked response was either much weaker or disappeared completely. Furthermore, if there was a response under this condition, it could only be elicited over a much more restricted region of the visual field. In all cells under both conditions, the best response always occurred when the stimulus was presented at the center of gaze, i.e., projected on the fovea of the retina. Many of the characteristics of the neuronal responses to the stimulus during the "blink" of the fixation spot were similar to responses obtained previously by others in the lightly anesthetized, immobilized preparation.

In summary, we have found that the behavioral context in which a visual stimulus is presented is important in determining the response of neurons in inferior temporal cortex. If the probe stimulus is presented while the animal is fixating another visual target, no matter how small, the response to the probe stimulus is either attenuated or absent. From these experiments alone we are not yet in a position to say whether the lack of responsiveness during fixation is due to some interaction among the stimuli within the visual system, or whether the visual attention of the animal to the fixation spot somehow interferes with the neuronal response to the receptive-field probe stimulus. Experiments are now under way to begin to sort out these alternative possibilities.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Solving the problem of how the brain processes sensory information has been a major goal of psychologists and neurophysiologists. These experiments are an attempt to move closer to an understanding of such processing in the visual system of the primate. Since the inferior temporal region is involved in higher-order visual processing, we expect that our investigations will not only further our knowledge of how this cortex codes and stores visual stimuli, but will also provide insight into visual perceptual and memory problems seen clinically as a result of acquired or congenital defects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02033-04 LN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Functional mapping of sensory systems

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2	1	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The [^{14}C] 2-deoxyglucose method was used to identify the cerebral areas related to vision through a comparison of glucose utilization in a visually stimulated as compared to a visually deafferented hemisphere in the rhesus monkey. Cortically, the visually related areas included the entire expanse of striate, prestriate, and inferior temporal cortex as far forward as the temporal pole, the posterior part of the inferior parietal lobule, and the prearcuate and inferior prefrontal cortex; subcortically, in addition to the lateral geniculate nucleus and superior colliculus, visually related structures included large parts of the pulvinar, caudate, putamen, claustrum, and amygdala. These results, which are consonant with a model that postulates an occipito-temporal-prefrontal pathway for object vision and an occipito-parieto-prefrontal pathway for spatial vision, reveal the full extent of those pathways and localize their points of contact with limbic, striatal,and diencephalic structures.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Converging evidence from studies of lesion effects, anatomy, and single units indicates that, in the primate, the system for processing information about visual objects extends beyond the striate cortex to include the circumstriate and inferior temporal areas. From the inferior temporal area, in turn, information about these objects appears to be transmitted to subcortical structures in the temporal lobe and to inferior prefrontal cortex. The same classical mapping techniques have also been used to identify another system, specialized for processing information about spatial vision, which also depends on a corticocortical pathway, in this case from striate through prestriate to inferior parietal and dorsal prefrontal cortex. In addition, both systems are known to have subcortical inputs to, and projections from, structures in the tectofugal pathway. Despite a range of research efforts, however, the full extent and precise boundaries of these cortical pathways are still undefined, as are their exact points of contact with their subcortical forebrain targets. The [^{14}C] 2-deoxyglucose method for measuring local cerebral glucose utilization (LCGU) has provided a means of clarifying these issues.

Experiment 1

To map the functional visual system by means of the 2-deoxyglucose technique, we prepared monkeys with a unilateral optic tract section combined with section of the forebrain commissures, thus visually deafferenting one hemisphere while leaving the other intact. This made it possible to compare LCGU values in a "seeing" and a "blind" hemisphere within the same animal, and thereby map the visually related areas.

Monkeys in this initial study sat in a primate chair while visual patterns mounted on a drum rotated around their heads. They were injected with a pulse of radioactively-labeled 2-deoxyglucose and monitored over the next 45 minutes to determine the levels of free deoxyglucose in the blood. Most of the radioactively-labeled substance is incorporated into active cells during this period, and any extracellular deoxyglucose is of sufficiently low concentration that its presence does not contaminate the autoradiographs.

In a second study, monkeys prepared surgically as above were trained on a visual discrimination task which required them to use the hand opposite the blind hemisphere. The task was a pattern discrimination in which a positive stimulus was paired with one of a series of negative stimuli in sequential blocks of trials. Correct responses were reinforced with a water reward.

In both behavioral situations, reduced glucose utilization in the blind as compared with the seeing hemisphere was seen cortically not only in the geniculostriate system, but throughout the entire expanse of circumstriate and inferior temporal cortex as far forward as the temporal pole and reaching even to the inferior prefrontal cortex. The functionally depressed zone included tissue adjacent to the inferior temporal cortex in the upper bank of the superior temporal sulcus and in the fusiform and perirhinal areas. Subcortically in the temporal lobe, side-to-side differences were seen in lateral

and dorsal amygdala, ventral putamen, ventral claustrum, and tail of caudate. Outside this stimulus-processing system specialized for object vision, asymmetries were also seen in the inferior parietal lobule and prearcuate region of the frontal lobes, the tissue specialized for spatial vision. Performance on the discrimination task led to an asymmetrical increase of glucose utilization in structures associated with the active hand and to a symmetrical increase in structures associated with the act of drinking; within the visually related areas, however, the effects of the two different testing situations were substantially the same.

The data from this experiment have now been examined in greater detail in order to delineate the exact borders of visually-related tissue in the parietal and temporal lobes. Within the parietal lobe, the ventral visual-nonvisual (VN) border first appears in the fundus of the lateral fissure (la) and then moves out of la along the cortical surface to the posterior lip of the intraparietal sulcus (ip). The dorsal VN border, which remains on the anterior bank of ip, gradually descends to the fundus. The two borders merge within ip approximately 5mm behind its anterior tip to form the rostral limit of visually related parietal cortex.

Within the temporal lobe, the dorsal VN border first appears at the fundus of la and moves quickly to the anterior lip and then halfway down the anterior bank of the superior temporal sulcus (ts). It remains at this depth through the posterior half of ts and then descends gradually to the fundus of ts. The ventral VN border moves gradually from the calcarine to the hippocampal fissure (h). It continues along the posterior half of h, moves laterally to the medial lip of the occipitotemporal sulcus, and then medially to the fundus of the rhinal sulcus. The dorsal and ventral borders merge on the ventral surface just in front of the anterior tip of ts to form the rostral limit of visually related temporal cortex.

Within visually related cortex metabolically different subareas could be delineated, as in the lower bank of ip and the upper bank of ts. Both VN and subareal borders were frequently sharp and consistent among animals. Preliminary analyses suggest that these borders separate architecturally different areas, lending new functional validity to architectonics.

Experiment 2

Portions of each of the visual areas within the cortical pathway serving object vision are known to be reciprocally connected through the forebrain commissures. In particular, the representation of the vertical meridian at the OC-OB border as well as selected parts of area OA receive commissural inputs via the splenium of the corpus callosum, while extensive portions of areas TEO and TE receive their contralateral input via the splenium and anterior commissure. Since the transfer of visual information between the hemispheres is critically dependent on these reciprocal connections, we attempted to localize and to quantify the contribution to vision made by the commissural systems. To do this we measured LCGU throughout the cortical visual system in two different surgical preparations: unilateral optic tract section combined with forebrain commissurotomy, and unilateral optic tract

section alone. The 2-deoxyglucose method was applied one month postoperatively in awake rhesus monkeys actively viewing visual patterns. The commissural contributions to vision were inferred from differences in LCGU between the deprived hemispheres of the two groups.

From the autoradiographs of each brain, representative sections were chosen at 1-mm intervals throughout the extent of the cortical visual pathway. Weighted averages of LCGU for the entire extent of each visual area were then calculated from these sections by means of a computerized image-processing system. In the intact hemisphere there was a progressive decline in LCGU along the cortical visual pathway from a high of 66 in area OC to a low of 47 μmoles/100g/min in anterior TE. This sequential decline in the intact hemisphere was the same both for the animals with tract section plus commissurotomy and for those with tract section only. There were also no differences between operated groups in the visually deprived hemisphere for areas OC through TEO, where LCGU averaged 50% of that in the intact hemisphere (ranging from 40% in OC to 60% in TEO). A difference attributable to visual input via the intact commissures was found in TE, however, where LCGU in animals with combined tract section and commissurotomy remained at 60% of that in the intact hemisphere, whereas in animals with tract section only LCGU reached 80% and 90% of the values in the intact hemisphere for posterior and anterior TE, respectively. These results indicate that commissural inputs contribute more to the visual functions of area TE than to those of any other visual area and that, in fact, commissural inputs alone may be insufficient to support visual function in areas TEO, OA, and the OC-OB border.

Experiment 3

The results of Experiment 2 have presented us with a paradox. The rise in metabolic activity in the deprived hemisphere of the tract-cut preparations clearly reflect the functional contribution of the forebrain commissures in the anterior portion of the occipito-temporal pathway but, surprisingly, not in the posterior portion. Thus, on the one hand, our metabolic data on area TE are in good accord with existing anatomical data, which indicates that area TE receives interhemispheric projections through both the splenium and the anterior commissure. On the other hand, substantial commissural fiber projections also reach the more posterior visual areas - specifically, the OC-OB border, OA, and TEO. Yet our metabolic data give no indication of this heavy and widespread projection. A possible explanation of this paradox is that, unlike the commissural input to area TE, which clearly can support visual function by itself, the commissural input to the more posterior zone may be effective only against a background of activity provided by an intact retino-geniculo-cortical pathway.

In order to test this hypothesis, we have prepared a series of monkeys in which a "blind" right hemisphere was produced by midline section of the optic chiasm combined with occlusion of the right eye rather than by right optic tract section. Through a comparison of LCGU in the right hemispheres of these animals and of those done previously with right optic-tract section, the functional effectiveness of commissural input with and without spontaneous retinal input may be evaluated. Greater metabolic activity in the former case

will indicate that the commissural fibers to the prestriate-posterior temporal zone do indeed require a minimum level of background activity from the intact retina in order to make a functional contribution to vision. We have completed the experiment with two animals and are awaiting the processed tissue for data analysis.

Experiment 4

In order to trace the functional development of the visual system, we are conducting studies in infant monkeys similar to those described in the first experiment. Thus far, a series of animals with unilateral optic-tract section combined with forebrain commissurotomy have been tested at various ages ranging from 2 days to six months. Preliminary examination of the data suggest that, metabolically, the visual system is almost fully developed by six months but that prior to this time different parts of the system reach maturity at different times. Quantitative analyses will be undertaken soon to trace the developmental sequence.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Since energy metabolism and functional activity are highly correlated within the nervous system as demonstrated by these and other studies, the 2-deoxyglucose method can provide a deeper understanding than has been possible before of the role of various cerebral structures in behavior. Our goal is to apply this method to the study of a variety of behavioral processes in the monkey, including perception, attention, memory, emotion, and volition, for the purpose of identifying the structures involved and quantifying the degree of their participation. Such understanding is certain to contribute greatly to the ultimate goal of diagnosing and treating a wide variety of neurological and mental disorders.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02034-03 LN

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Subcortical mechanisms related to frontal lobe functions in the monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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2	1.5	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The dopamine-containing neurons of the substantia nigra project to the dorsolateral prefrontal cortex and the striatum. In the rhesus monkey electrolytic lesions of the prefrontal cortex or the related parts of the striatum, or depletion of dopamine in the prefrontal cortex, impair performance of delayed alternation, a test of cognitive function. However, bilateral destruction of the substantia nigra in a pilot animal had little, if any, effect. It therefore becomes important to establish the generality of this negative finding, and to explore what significance for behavior there is in the biochemical relationships of these DA-containing structures.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

The purpose of this study is to examine the effects of lesions of the substantia nigra on delayed-alternation behavior and other cognitive functions in the monkey. This has become important because of conflicting data concerning the effects of nigral lesions on cognitive functioning.

Delayed alternation, a test of cognitive function, is severely impaired in monkeys by selective lesions of the dorsolateral prefrontal convexity, or of those portions of the striatum to which it projects. The dopamine (DA)-containing neurons of the substantia nigra send a powerful projection to the striatum. A similar, and fairly selective, projection to the dorsolateral prefrontal convexity has recently been demonstrated as well. Depletion of DA in this cortical region with the neurotoxin 6-hydroxydopamine impairs delayed-alternation performance in the monkey and lesions of the DA-containing neurons of the substantia nigra and surrounding regions impair it in the rat. It therefore seemed likely that destruction of the substantia nigra in the monkey would also disrupt delayed-alternation behavior. Surprisingly, this has not turned out to be the case.

Lesions were aimed at the substantia nigra by combining stereotaxic and x-ray techniques to guide the approach in three animals. Each animal was retested on delayed alternation, and visual discrimination (a control task) and two were tested on a 24 hr. concurrent learning task to measure original acquisition of new information as well. A neurological test designed to detect deficits in motor and sensory function was also administered. Finally, two animals were challenged with haloperidol, a dopamine antagonist, and L-DOPA, a precursor known to increase dopamine levels, prior to retesting them on delayed alternation.

No convincing deficit was found in either delayed alternation, visual discrimination, or 24-hr concurrent learning. In initial neurological examinations following surgery, the animals appeared normal in motor ability, alert, and responsive to visual stimuli. There did appear to be some neglect of auditory, and, more especially, somatosensory input. Subsequent observations indicate these deficits may ameliorate with time. Neither haloperidol nor L-DOPA affected delayed-alternation performance reliably.

We are now preparing the brains for histological examination and for biochemical analysis of amine levels in prefrontal cortex and caudate nucleus, two primary targets of dopamine-containing substantia nigra neurons.

The negative findings on delayed alternation are puzzling, especially in view of the fact that a deficit was demonstrated for a similar task in rats. The neglect that was detected is indicative of nigral lesions, but our conclusions must remain tentative until the histological and biochemical analyses are completed.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Dopamine, a chemical transmitter manufactured particularly by neurons of the substantia nigra, has been heavily implicated in schizophrenia. The portion of the cerebral cortex that receives the heaviest input of dopamine is necessary for the performance of various attentional, mnemonic, and spatial tasks, among them the ability to perform delayed alternation. The nature of the dependence of the cortex on its dopamine inputs is as yet unknown, as is the precise relationship of dopamine to mental health and disease. It is the purpose of this project to provide such information, which eventually could be of both diagnostic and therapeutic use.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02035-01 LN

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Anatomy of the primate visual system

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L.G. Ungerleider	Senior Staff Fellow	LN NIMH
OTHER:	M. Mishkin	Act. Chief, Laboratory of Neuropsychology	LN NIMH
	R. Desimone	Staff Fellow	LN NIMH
	B.J. Richmond	Medical Officer, Research	LN NIMH
	C.G. Gross	Professor	Princeton Univ.
	R. Gattass	Fogarty Fellow	Princeton Univ.
	A.P.B. de Sousa	Fogarty Fellow	Princeton Univ.

COOPERATING UNITS (if any)

Princeton University

LAB/BRANCH

~~Laboratory of Neuropsychology~~

SECTION

Section on Cerebral Mechanisms

INSTITUTE AND LOCATION

~~NIMH, NIH, Bethesda, Maryland 20205~~

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The sensory processing and perception of visual information requires the transmission of neural activity across a corticocortical, multisynaptic pathway from striate cortex, or area 17, through several prestriate "association areas". In addition, visual input from striate cortex reaches these prestriate areas indirectly via two subcortical visual structures, the superior colliculus and the pulvinar. We have begun to explore the complex circuitry and visuotopic organization of these connections in the visual system of the macaque monkey by the combined use of anterograde degeneration and autoradiographic tracing techniques. Our results have demonstrated direct striate projections to three separate prestriate areas, each of which contains a re-representation of the visual field. Corticocortical efferents of these prestriate projection areas are now being examined to determine the pathways by which visual information is relayed from the occipital lobe to the still higher-order visual areas located within the parietal and temporal lobes, visual areas that are critical for spatial vision and object vision, respectively.

PROJECT DESCRIPTION:

Striate cortex, the primary visual cortex, is the source of two major corticocortical, multisynaptic visual pathways. One of these follows the course of the superior longitudinal fasciculus, interconnects the striate, prestriate, and posterior parietal areas, and appears to be important for spatial vision. The other follows the course of the inferior longitudinal fasciculus, interconnects the striate, prestriate, and inferior temporal areas, and is critical instead for object vision. Although visual information must reach the posterior parietal and inferior temporal cortex to enable their participation in spatial vision and object vision, respectively, the complex circuitry through which this information is transmitted has yet to be unraveled. We have undertaken to examine the details of the connections within these two cortical visual pathways, beginning with an analysis of the projections of the striate cortex itself.

Experiment 1: Anatomical material from two series of monkeys (Macaca mulatta) was used to determine the locus, extent, and visuotopic organization of striate projections to prestriate cortex. One series was processed for terminal degeneration by the Fink-Heimer procedure following unilateral lesions of lateral, posterior, or medial striate cortex, areas representing central, peripheral, and far peripheral vision, respectively. Collectively, the lesions included all of area 17 with little or no invasion of area 18. The second series was processed for autoradiography following tritiated amino-acid injections into various striate sites representing the center of gaze and eccentricities ranging from 0.5° to 45° in either the upper or lower hemifield. The results indicated that striate cortex (cytoarchitectonic area OC) projects to at least three separate and topographically organized visual areas within prestriate cortex: a circumstriate cortical belt which corresponds to area OB; the posterior bank and floor of the caudal portion of the superior temporal sulcus; and the depth of the posterior intraparietal sulcus.

Now that the total system of striate-prestriate projections has been delineated, the effects of completely disconnecting inferior temporal from striate cortex can finally be investigated. Accordingly, monkeys are being prepared with complete lesions of just the three striate projection fields described above (without inclusion of any other prestriate tissue) prior to testing them on visual pattern discriminations. In addition, we are trying to determine whether even small prestriate lesions might disconnect inferior temporal cortex from corresponding parts of the visual field by testing discrimination of stimuli confined to those parts of the field in monkeys that have been trained to maintain fixation. In view of the clear visuotopic organization of the striate projection zones in both area OB and the caudal portion of the superior temporal sulcus, even limited lesions within these prestriate zones should yield severe visual deficits provided the animals are forced to use the part of the visual field corresponding to the area damaged. To monitor fixation accurately, we are making use of a magnetic eye-coil system.

Experiment 2: Having delineated the projections of striate to prestriate cortex, which were found to consist of three separate re-representations of

the visual field, we are now following these projections further, with the inferior temporal and posterior parietal cortex as our targets. Since the multiple prestriate areas can be identified not only by their approximate location, but also by their distinct electrophysiological properties, all autoradiographic studies are now being performed under physiological control. By recording the activity of neurons from the microsyringe needle and mapping receptive fields, we are able to inject tritiated amino acids into portions of each prestriate area that represent known parts of the visual field. To date, this recording technique has been used for injections into both area OB and the caudal portion of the superior temporal sulcus, and the autoradiographic processing of the material is currently in progress. In addition to tracing the connections of the multiple prestriate cortical areas, we are attempting to develop a myeloarchitectural stain that will serve as a "marker" to distinguish among them. Ultimately, we hope to correlate the data from our connectional studies with those obtained by this histological procedure.

On the assumption that the two visual pathways from striate cortex can indeed be followed stepwise to the visual areas within the parietal and temporal lobes, a major question for the future will be how the spatial and object information carried in these two separate pathways are subsequently integrated anatomically to permit a unified visual percept.

Experiment 3: Anatomical material prepared for Experiment 1 was used to investigate subcortical efferents from striate cortex to the pulvinar, a nucleus in the thalamus implicated in attentional mechanisms. We had found in Experiment 1 that striate projections to the prestriate cortical area OB are visuotopically organized, and we had prior evidence of a similar topographic arrangement of pulvinar projections to area OB. In the present study, we found that there is also a precise visuotopic organization of striate projections to the pulvinar, indicating the existence of two sources of striate input to area OB that are in perfect register: one, direct, i.e., corticocortical; and the other, indirect, via the pulvinar. This parallel system of inputs to area OB thus provides a possible mechanism by which activating signals (e.g., from dorsal cortical areas to the pulvinar via midbrain structures) acquire visual field specification, that is, a mechanism that tells the organism where in the field to attend. Although this hypothetical circuit may apply for all sensory systems, for the visual modality our data indicate that only the inferior and lateral nuclei of the pulvinar are involved. Future anatomical studies will investigate the sources of midbrain input to the inferior and lateral pulvinar, as well as the organization of afferents to the pulvinar from other sensory modalities. In addition to the anterograde tracing techniques of degeneration and autoradiography, these studies will employ horseradish peroxidase for tracing retrograde axonal transport.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

An understanding of the basic mechanisms mediating normal vision is the first step in the prevention, diagnosis, and alleviation of sensory, perceptual, and mnemonic disorders. In particular, a delineation of the neural circuitry involved in the transmission of visual information beyond the striate cortex

may promote advances in the treatment of residual visual function following injury, by either disease or acute assault, to the central visual pathways. To this end, we have begun to explore the projections of striate cortex, both to prestriate "association areas" and to subcortical visual structures. Our ultimate goal is to unravel the complex system of projections to the still higher-order visual areas located within the parietal and temporal cortex, areas critical for spatial vision and object vision, respectively. The combined use of degeneration and axonal transport techniques provides a powerful tool for tracing neural connections within these central visual pathways.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02036-01 LN

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neural coding of visual stimuli in the immobilized monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Desimone	Staff Fellow	LN NIMH
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	C.G. Gross	Professor	Princeton Univ.
	M. Misnkin	Act. Chief, Laboratory of Neuropsychology	LN NIMH

COOPERATING UNITS (if any)

Princeton University, New York University Medical School

LAB/BRANCH
Laboratory of Neuropsychology

SECTION
Section on Cerebral Mechanisms

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER X

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The neural mechanisms for the visual recognition of objects extend beyond striate cortex into the surrounding prestriate and inferior temporal visual areas. Neurons in the prestriate visual areas appear to process local properties of objects such as the location of boundaries, while inferior temporal neurons appear to process more global features such as object shape. We are studying single neurons in prestriate and inferior temporal cortex to investigate both mechanisms. In inferior temporal cortex we found that over half the neurons were tuned to a set of shape descriptors that can be used to code object shape. Since different neurons are tuned to different descriptors, a population of inferior temporal neurons could code any shape. We are comparing this tuning for object shape to tuning for spatial frequency gratings, since it has been shown that such gratings can be used to represent the retinal image. In prestriate cortex, we have begun our recording studies in area MT. We have found that MT has a vertical columnar structure for processing the axis of stimulus motion.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Previous work in this and other laboratories has shown that the cortex located beyond the primary visual cortex, or striate cortex, contains many additional visual areas. These extrastriate visual areas complete the process of pattern recognition begun in striate cortex and supply the motor systems and the limbic affective and motivational systems with visual information. In this project we are studying the role of extrastriate visual neurons in perception and memory. In order to study the passive visual properties of extrastriate neurons, unaffected by eye movements or the changing state of the animal, we are recording neural activity in the immobilized, lightly anesthetized macaque. In the immobilized preparation we have complete control over the pattern of stimulation on the retina, and, because the monkey does not move, we can study individual neurons for many hours.

Experiment 1: Shape Classification by Inferior Temporal Neurons

The inferior temporal visual area (IT) of primates is known to play an important role in visual perception and memory. We have shown previously that, unlike neurons in striate or prestriate cortex, IT neurons have large receptive fields which include the center of gaze and are not retinotopically organized. Furthermore, while striate and prestriate neurons are sensitive to local properties of visual objects, such as the location and orientation of an edge, IT neurons appear to be sensitive to the global properties of complex objects, such as their shape. Therefore, we have begun the study of global feature coding by IT neurons by studying shape analysis.

Following image segmentation, i.e., finding the boundaries of objects on the retina, the discrimination of object shapes is one of the most fundamental problems of pattern recognition. The problem is to assign an object to one of many object classes based on a set of measurements of the object's shape. It is well known from the machine pattern-recognition literature that an extremely important measure of shape is boundary curvature. Although a variety of measures of boundary curvature have been proposed, a particularly potent measure is the Fourier descriptor.

The Fourier descriptors are functions that can be used to generate any arbitrary closed figure. Likewise, any closed figure can be decomposed into a unique set of these shape descriptors. Since shapes that look similar have Fourier descriptors of similar value, the descriptors are a very powerful method for classifying or recognizing shapes. In addition, the computation of the Fourier descriptors is relatively simple. Briefly, the boundary of an object is represented by plotting the tangent to the curve as a function of arc length around the boundary. This periodic function is then decomposed into a sum of sinusoidal components, the Fourier descriptors, that vary in amplitude and phase. Thus, just as complex sounds can be represented by the sum of sinusoidal tones, complex shapes can be represented by the sum of sinusoidal "visual tones" (the descriptors) that wrap around the boundary. What do these pure "visual tones" look like when represented as closed curves? The 0th term Fourier descriptor is a circle. Higner order, nth terms, look like circles which have been pushed in to create n lobes. The amplitude of the descriptor is reflected in the depth of the lobes.

How might inferior temporal neurons decompose shapes into their Fourier descriptors? We propose that the initial stages of the decomposition are performed in striate and prestriate cortex. It is well known that striate and prestriate neurons are sensitive to the location and orientation of luminance contours; thus, they provide the tangents to the object boundary. Indeed, one of the functions of prestriate cortex may be to "break camouflage" and find the object contours based on properties such as texture, color, and movement. In any case, since inferior temporal neurons receive inputs from a wide region of prestriate cortex, they could determine the periodicity (sinusoidal components) of the boundary representation in prestriate cortex. If different IT neurons were selective for different sinusoidal components of the boundary representation, they would, in effect, function as the Fourier descriptors.

To test the hypothesis that inferior temporal cortex decomposes shapes into their Fourier descriptors (or some similar shape descriptor), we have begun to study the responses of IT neurons to closed curves that, when decomposed, are pure Fourier descriptors. We typically test each neuron with an octave series of terms (frequencies 2 through 64) and at different amplitudes and sizes.

Although the data are still preliminary, we have tested over 150 IT neurons, and over half have exhibited tuning curves with a peak at one of the Fourier descriptors. Furthermore, we have replicated the shape of the tuning curves when we have tested the same units with different sizes and amplitudes of the basic shape series. Clearly, much additional work will be needed to determine if some IT neurons actually decompose shapes into their Fourier descriptors, but our initial data are extremely encouraging that we are at least on the right track.

Experiment 2: Spatial Frequency Selectivity of IT Neurons

Just as an object's shape can be represented as a sum of sinusoidally modulated curves, an entire retinal image can be represented as a sum of oriented sinusoidal contrast gratings (The gratings resemble out-of-focus jail-cell bars.). The set of sinusoids that represent the image is known as its two-dimensional Fourier transform, and it has been frequently suggested that this transform is the basis on which the visual system recognizes objects. Although we favor other approaches to pattern recognition, it is conceivable that IT neurons do decompose the retinal image into its oriented sinusoidal components. Therefore, we have begun to measure IT neuronal responses to sinusoidal frequency gratings and to compare these with their responses to the shapes described by the Fourier descriptors (Experiment 1).

Experiment 3: Organization of Directionally Selective Cells In Area MT

Visual area MT, also known as the "motion selective" area, is a prestriate visual area in the macaque. Relative to other visual areas, MT has a high proportion of cells that are selective for the direction of stimulus movement but are relatively nonselective for stimulus form or color. Previous work in this laboratory has shown that MT receives topographically organized projections from striate cortex. Although MT does not project directly to inferior temporal cortex, our preliminary data suggest that it may do so

indirectly. Thus, MT may provide IT with information about object boundaries based on common movement. To study the organization of directional selectivity in MT, we recorded from single neurons isolated every 50 microns in penetrations throughout MT. Projected spots or slits were moved on a tangent screen and the optimal direction and axis of stimulus movement determined for each cell. (Axis of movement is the orientation of the linear path defined by a moving stimulus and varies from 0 to 180 degrees.)

Among 458 units recorded on 14 penetrations in three animals, 81% responded optimally to one direction of movement. In long tangential or oblique penetrations, the preferred axis of movement of cells changed smoothly and systematically. By contrast, there were few long systematic progressions of optimal direction of movement because of frequent 180-degree reversals. The rate of change of optimal axis of movement was, on the average, greater along penetrations approximately parallel to the cortical surface than along penetrations normal to the surface. These results suggest a vertical ("columnar") organization of axis of movement in MT.

The representation of axis of movement in MT is similar to that of orientation in striate cortex. For both, a full 180-degree cycle is represented in about 0.5mm of cortex. Furthermore, just as the ocular dominance system is superimposed on that for orientation in striate cortex, in MT there may be a direction-of-movement system that is superimposed on that for axis of stimulus movement.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

The primate, including man, is a very visual animal. Thus, it is not surprising that perhaps half of the primate cerebral cortex is involved directly or indirectly in visual processing. Consequently, the study of neural mechanisms of vision has proven to be not only of fundamental importance for our understanding of visual perception and memory but also for our understanding of brain function in general. The extrastriate visual areas described in this project are of particular importance to the mental health field because they are the source of nearly all the visual information for the limbic affective and motivational systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00471-26 LPP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Heredity and Environment in Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David Rosenthal	Guest Worker	LPP NIMH
OTHER:	Allan F. Mirsky	Chief	LPP NIMH
	Edward K. Silberman	Clinical Associate	LPP NIMH
	Paul H. Wender	Professor of Psychiatry	University of Utah
	Seymour S. Kety	Professor of Psychiatry	Harvard University
	Shmuel Nagler	Research Psychologist	Institute for Research on Kibbutz Education (Israel)
	Fini Schulsinger	Co-Director	Psychological Institute (Denmark)
	Olive W. Quinn	Guest Worker	LPP NIMH
	Patricia Lowing-Gala	Staff Psychologist	William Beaumont Hospital
	Arje Latz	Associate Professor	Boston University

COOPERATING UNITS (if any)

Harvard University, Institute for Research on Kibbutz Education, University of Utah, Psychological Institute (Denmark), and William Beaumont Hospital, Michigan

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is composed of the following studies: (1) An intensive multi-disciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. (2) Studies of adoptees and their biological and adoptive families. (3) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

PHS-6040
(Rev. 10-76)

Project Description:

The project is composed of the following studies: (1) An intensive multidisciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. We are continuing our contacts with this family to see what happens in the clinical course of these women and to see how the course is related to earlier and to current life experiences; (2) Studies of adoptees and their biological and adoptive families in Denmark; (3) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

The objectives of this project are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

The analysis of the literature has been reported in a series of papers and in an earlier published study of the quadruplets. A textbook which organizes and explicates the literature on the genetics of behavioral disorders and a modified version of this book has also been published. The studies of the Danish adoptees indicate that hereditary factors contribute to the development of schizophrenia and to other disorders that we include in a group called the schizophrenia spectrum. Several articles are in preparation, and eventually a monograph will be written on the adoption studies. Data analysis is continuing. One hundred subjects in the Israel study have been examined, and the research findings are currently being analyzed and evaluated. Preliminary findings indicate that offspring of schizophrenics have more neuropathological signs, abnormal psychiatric signs, and impaired cognitive functions in childhood than do controls. Six or more subjects have had a breakdown, and all have a schizophrenic parent.

The proposed course of this project is to (1) reexamine the quadruplets with all the modern armamentarium of investigative tools of neuropsychobiology and biological psychiatry. The women and their relatives have been brought to NIMH and their examination will have been completed by the end of June 1981. (2) to finish the write-up and publication of the psychological studies of the Danish adoptees. The Danish adoption data are being analyzed by Dr. Patricia Lowing-Gala at the Beaumont Hospital in Royal Oak, Michigan. Her participation is covered by a Professional Services order; she is in consultation with the coprincipal investigators of the original study, and in particular with Drs. Wender and Kety. (3) to publish the existing data on the 100 Israeli cases and to reexamine them, in Israel, for their current level of psychopathology. This will allow a reasonably final statement concerning which of the measures made in childhood predict psychopathology in young adulthood.

The subjects are to be interviewed and examined in Israel by Dr. Arje Latz of Boston University under a Professional Services order. As an Israeli and a trained clinician, he is especially suitable to conduct this work. It will

be done with the close collaboration and good offices of Dr. Shmuel Nagler of Haifa University and The Oranim Teachers College.

In press:

Rosenthal, D.: The Genetic Environmental Perspective in Psychopathology. In Al-Issa, I. (Ed.): Culture and Psychopathology. Baltimore, University Park Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00472-13 LPP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Investigations of Some Formal Characteristics of Speech

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Theodore P. Zahn	Research Psychologist	LPP	NIMH
Other:	Donald S. Boomer	Guest Worker	LPP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This research project investigates attention during natural speech perception. Specifically, we are attempting to test the hypothesis that speech perception involves phasic, rather than continuous attention and is characterized by bursts of cognitive activity at linguistically specified boundaries in the stream of speech. The method involves the measurement of reaction time to irrelevant stimuli--clicks--while the subject is listening to a tape-recorded dialogue. If the hypothesis is correct, the responses should be relatively inhibited for those clicks timed to occur during the postulated bursts of cognitive activity at linguistic boundaries. If the fundamental hypothesis can be given empirical support, this method will be used to investigate attention deficit in schizophrenia.

This project has been temporarily inactive.

PHS-6040
(Rev. 10-76)

Project Description

The guiding hypothesis of this study, growing out of work on listener responses is that listeners, like speakers, process speech in "chunks," or temporal patterns, the chunks being phonemic clauses. If the decoding process operates with phonemic clause speech units, then attention to the incoming speech need not be continuous. We hypothesized that attention would be maximally occupied at the end of the phonemic clause and minimally occupied while the clause is being accumulated and stored.

In the present study, subjects listen to a rather absorbing tape recorded dialogue in one ear while also attending to an irregular series of clicks presented to the other ear. They respond to each click by depressing a telegraph key as quickly as possible. Reaction times are measured and stored. Half of the clicks are precisely timed to occur at the end, or terminal juncture, of phonemic clauses. Our hypothesis is that the subjects' attention to the cognitive demands of speech decoding will be different during clauses than at the ends of completed clauses, and this difference wil be reflected in the mean response latency to the two sets of linguisticically-specified clicks.

We found that for right-handed subjects, when speech is presented in the right ear and clicks in the left, reaction times were about 20 msec. faster to clicks at the terminal juncture than to those during the clause (18 of 20 subjects showed this effect). The data suggest that fluctuations of attention do occur during speech perception, but contrary to the original hypothesis, speech processing seems to be more active during a clause than at the terminal juncture. During the past year we completed testing of 20 right-handed subjects, but with speech going to the left ear and the clicks to the right ear, and have tested a number of left-handed subjects on the original speech--right ear mode. Most of these subjects also show faster reaction time at the terminal juncture but to a somewhat lesser degree than the original group. However, left-handed males seem not to show any clear effect of click placement. We will continue to try to recruit more left-handed males to complete this group.

Beyond the present normative studies lie the possibilities of investigating attentional abnormalities or deficits in schizophrenia, with particular attention to the specification of hemispheric asymmetry in cortical function. It is widely believed that cognitive and perceptual deficit in schizophrenia may reflect an underlying disorder of attention, specifically the ability to extract the salient elements of complex stimulus patterns. Since speech is just such a complex pattern and since the hemispheres are asymmetrically specialized for speech, a psycholinguistic approach to the problem of laterality and attention seems to be both neglected and promising.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00484-21 LPP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Psychophysiological Responsivity and Behavior in Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Theodore Zahn	Research Psychologist	LPP	NIMH
OTHER:	Carmi Schooler	Research Psychologist	LSES	NIMH
	Monte Buchsbaum	Research Psychiatrist	BPB	NIMH
	Dennis Murphy	Chief	CNB	NIMH
	Daniel VanKammen	Research Psychiatrist	BPB	NIMH
	Thomas Robinson, Jr.	Guest Worker	LPP	NIMH
	Suzanne Haynes	Epidemiologist	HV EP	NHLBI
	Larry Siever	Clinical Associate	CNB	NIMH
	Thomas Insel	Clinical Associate	CNB	NIMH

COOPERATING UNITS (if any)

Laboratory of Socio-Environmental Studies, Biological Psychiatry Branch, Laboratory of Clinical Science, and Ward 4-East

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Laboratory of Psychology and Psychopathology

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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	0.8	0.9

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The general purpose of this project is to investigate the roles of autonomic nervous system activity, attention, and information processing and their interrelationships in the pathology, etiology and prognosis of psychiatric disorders. A second purpose is to determine biological and psychological processes related to ANS activity as assessed by peripheral measures such as skin conductance, heart rate and skin temperature. Subjects with diagnoses of schizophrenia, depression and neurosis are tested under conditions of rest, presentation of tones and performance on reaction time, mental arithmetic, two-flash discrimination or tachistoscopic recognition tasks. Biological mechanisms influencing ANS activity and attention are investigated by testing the effects of drugs and other treatments and by correlating these variables with enzyme activity and levels of biogenic amines and their metabolites. Psychological determinants are investigated by correlating the results with personality, mood and personal history questionnaires, by information from interviews, and by the effects of procedural variations.

PHS-6040
(Rev. 10-76)

Project Description

A. Objectives

The major objective of this project is the further understanding of the role of autonomic nervous system (ANS) activity, information processing and attention and their interrelationships in psychiatric disorders, primarily schizophrenia. The overall strategy involves studies of ANS and attentional relationships to diagnosis and prognosis, studies of the effects of drugs and other therapeutic interventions, "high risk" and personality studies in normal volunteers and studies of the measurement of ANS activity.

B. Methods Employed

The general methods of these studies include measurement of ANS activity through skin conductance (SC) usually measured bilaterally, heart rate (HR), vascular activity (skin temperature and finger pulse volume) and respiration while subjects are resting, exposed to a series of nonsignal tones of constant or of variable intensity and performing tasks. Tasks include tests of attention using reaction time techniques, tests of perceptual speed using two-flash discrimination and tachistoscopic recognition and tasks designed to be moderately stressful. A minicomputer system is used to run the experiments and to collect and analyze the data. Studies in various stages of completion are listed below.

1. Schizophrenic Studies

a. A study with BPB (see Z01-MH 00132 BPB) of newly admitted, drug-free patients used a "balloon stress" (blowing up a balloon until it pops), and the two tests of perceptual speed given on different days. The tachistoscope task allows separate evaluation of ANS responses to stimuli differing in significance and to positive and negative reinforcements. This should allow testing of the hypothesis, developed in previous studies, that schizophrenics' ANS does not respond appropriately to variations in stimulus significance. This study also includes several rest periods and a series of nonsignal tones for comparative purposes.

b. In current studies ANS recording is being carried out in two sessions of rest, tone series and reaction time using the first method described below. In addition several methods of assessing attention deficits using reaction time (RT) techniques are being compared: (1) the classical "set" procedure of Shakow which involves variations in the foreperiod in a simple auditory RT paradigm, (2) RT to visual and auditory stimuli, measured when the stimuli are predictable, unpredictable or simultaneous (but unpredictably so). We have confirmed previous findings in normals that although RT to tones is faster than RT to lights when the stimuli are predictable, RT to light is faster under unpredictable simultaneous presentation. This is taken to indicate an attentional bias toward visual stimuli or visual dominance, (3) comparison of ipsimodal vs. crossmodal sequences of

tones and lights in a simple RT paradigm, plus occasional simultaneous presentation to assess "intersensory facilitation." Simple RT is faster under simultaneous presentation of a tone and light in the context of an unpredictable series presumably because the subject's response is triggered by whichever of the two stimuli he is attending can be tested.

c. Patients are being tested during their hospitalization using a procedure of resting, a series of variable intensity tones (60-100 dB) and a two-flash discrimination procedure. Patients in this study are on an active treatment or placebo. Drugs such as pimozide, lithium, naltrexone, GHB and propranolol and other treatments such as hemodialysis and plasmaphoresis are evaluated.

2. Studies on nonschizophrenic psychopathology.

a. Patients with depressive and obsessive compulsive disorders are being tested shortly after hospital admission or as outpatients (on a protocol identical to to the first part of the schizophrenics' protocol described in 1.b. above) in collaboration with the CNB. Patients are tested while being treated with tricyclic antidepressants and monoamine oxidase inhibitors as in 1.c. above.

b. In collaboration with BPB three cases of multiple personality have been tested in five sessions each on short versions of the rest, tones and reaction time procedure. The method is to repeat tests of different personalities in a different order each time to control for adaptation, and compare the between-personality variance.

3. Studies on normals

a. Two "high risk" studies in collaboration with BPB (Project 0035 BPB) in which subjects are selected on the basis of performance on attention tasks, have been carried out. In one, subjects were selected for very good or very poor performance on the Continuous Performance Task, and in the other, pendulum eye-tracking was used. The procedures we have used were similar to those used in the current studies on schizophrenia.

b. Two studies of reactions to physical and psychological stress have been done in collaboration with LCS and BPB. In addition to ANS measures, measurement of changes in norepinephrine, B-endorphin and cortisol from plasma have been made.

c. In collaboration with LSES (Project Z01-00674 LSES) a method of confirmatory factor analysis is being used on ANS and personality data from 95 normal subjects. This has the objective of developing error-free measurement models of the structures of these systems, in order to reduce the large number of variables generated by the ANS and personality assessment procedures to many fewer and more "pure" concepts. This method may lead to causal models of the interrelationships between systems.

C. Major Findings

1. Schizophrenic Studies

a. In a completed study we showed that a pattern of ANS and attentional functioning--high ANS "arousal," small ANS responses particuarly to meaningful or demanding stimuli and situations, slow adaptation and habituation and poor attention--characterized unmedicated acute schizophrenics compared to normal controls, and within the schizophrenic group, predicted a poor clinical outcome of a 4-month hospitalization. Preliminary analysis of the data has partially confirmed the previous study in finding high ANS activation, low reactivity and slow adapation in patients compared to controls. An anomalous finding in the previous study--that skin conductance level, a commonly used index of ANS activation, differed from the other indices in being slightly lower in the schizophrenics--was also partially confirmed in that it was quite similar in the two groups.

b. Data are still being collected, but it is apparent that the phenomena of visual sensory dominance and intersensory facilitation found in normal subjects also occur in schizophrenics.

c. Hemodialysis treatment (compared to sham dialysis) although having no significant clinical effects, produced an exaggerated ANS response to novel stimuli, especially a 100dB tone, suggesting that this treatment may have removed something from the system that normally inhibits ANS reactions to novel and/or aversive stimuli. Analysis of pharmacological effects are being started.

2. Studies on nonschizophrenic psychopathology

a. No reportable findings as yet.

b. Multiple personality subjects are being evaluated on a case-by-case basis. Preliminary analyses of the data suggest consistent ANS differences in some personalities within subjects and that habituation of the skin conductance orienting response in one personality may be unaffected by the prior experience of another personality.

3. Studies on normals

a. Poor attenders, as defined by the CPT, were found to have increasingly impaired reaction time as the amount of processing required increased from simple (20-25 msec) to choice reaction time (100 msec). This deficit seems due to a problem of shifting attention--from longer to shorter foreperiods in the Shakow procedure or between stimulus modalities in the other procedures. Thus we have found a rather specific attention deficit in normal subjects that appears on several tests and is similar in kind to what has been found in psychopathology.

b. In both stress studies it has been found that plasma norepinephrine increases to physical stress but not to psychological stress. Other purported indices of ANS activity, notably skin conductance and heart rate, change markedly to both types of stress. This suggests relatively greater peripheral control of norepinephrine and relatively greater central control over the other measures. Coronary prone (or "Type A") middle aged men surprisingly evidenced smaller increases in norepinephrine and heart rate to physical (postural change from supine to standing) than Type B men.

c. Measurement models have been obtained successfully for concepts of skin conductance tonic arousal, skin conductance lability, skin conductance response speed and heart rate arousal for males and females in two different testing sessions, and for concepts of activity and mental health derived from a large number of personal history and personality variables. The strongest relationships found indicate that for women activity is negatively correlated with cardiovascular activity and for both genders perceptual speed is positively corelated with skin conductance indices of ANS arousal. Platelet monoamine oxidase activity correlates negatively with active behavior and positively with skin conductance activity.

D. Future Course of this Project

Analysis will continue of data for the completed project on schizophrenia with the goals of determining the relationship of ANS variables to diagnosis, diagnostic subtype symptomatology, severity of psychosis, performance on tests of attention and perceptual speed, degree of improvement during hospitalization and improvement on specific treatments. ANS activity in schizophrenia will be studied in relation to data obtained from biochemical assays of body fluids such as monoamines and their metabolites in CSF and monoamine oxidase activity. Predictors of clinical change after amphetamine infusion will be sought.

Collection of data will continue for current projects on schizophrenia, and nonschizophrenic psychopathology. It is hoped to broaden the diagnostic comparisons by testing patients with severe anxiety neuroses. Normal controls will be tested on the same protocol. This protocol will be used in the collaborative LPP project on attention disorders.

Investigation of ANS and behavioral effects of various pharmacological therapeutic agents will continue for all these groups. Statistical analysis will be done for those with adequate data with the purposes of determining the general effects of the drugs and correlates with clinical response.

Data analysis will be completed on the high risk projects for group comparisons and correlation with other data on the same subjects. We will complete the general model of ANS task performance, personality, and mood

variables to attempt to model other features of the data such as adaptation and habituation. If these methods prove useful we will apply them to other data bases for both patients and normal subjects.

E. Significance to Biomedical Research and the Program of the Institute.

Investigations of ANS activity and attention in psychiatric disorders, especially schizophrenia, have produced promising results which suggest that these processes may play fundamental roles in the etiology and expression of the disorders. Limitations on inferences to be drawn from measures of ANS activity come from incomplete understanding of their biological and psychological determinants. One of the main goals of this research is to increase this understanding by investigations of biological and psychological correlates and improvising measurement techniques. Evidence of ANS effects of amphetamine in normal men which are similar to the ANS activity seen in unmedicated schizophrenics (see project #Z01 MH 00486-09) and similar effects of L-dopa reported in the literature permit the hypothesis that at least some aspects of ANS functioning may reflect the activity of dopaminergic systems. The dynamic nature of these measures permits the study of processes such as adaptation, habituation, response to and recovery from stress, and effects of single stimuli through noninvasive techniques. Thus, further understanding of their mechanisms could greatly increase their utility in investigations of psychopathology. Continued investigations of the diagnostic specificity of these processes and of their relationships to other clinical features and to prognosis are necessary to confirm and extend our previous results and to test the limits of their generality.

Publications

Zahn, T.P.: Predicting outcome from measures of attention and autonomic functioning. In Baxter, C. and Melnechuk, T. (Eds.): Perspectives in Schizophrenia Research. New York, Raven Press, 1980, pp. 81-104.

Zahn, T.P., Carpenter, W.T., Jr., and McGlashan, T.H.: Autonomic nervous system activity in acute schizophrenia: I. Method and comparison with normal controls. Arch. Gen. Psychiatry. 38: 251-258, 1981.

Zahn, T.P., Carpenter, W.T., Jr., and McGlashan, T.H.: Autonomic nervous system activity in acute schizophrenia: II. Relationships to short term prognosis and clinical state. Arch. Gen. Psychiatry. 38: 260-266, 1981.

Zahn, T.P. Autonomic nervous system markers of diagnosis and prognosis in schizophrenia. In Hanin, I. and Usdin, E., (Eds.): Biological Markers in Psychiatry and Neurology. London, Pergamon Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00486-09 LPP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Psychophysiological Concomitants of Minimal Brain Dysfunction in Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Theodore P. Zahn	Research Psychologist	LPP	NIMH
OTHER:	Judith L. Rapoport	Chief, Unit on Childhood Mental Illness	BPB	NIMH
	Robert Elkins	Clinical Associate	BPB	NIMH

COOPERATING UNITS (if any)
Biological Psychiatry Branch

LAB/BRANCH
Laboratory of Psychology and Psychopathology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many investigators believe that the autonomic nervous system (ANS) may be involved in hyperactivity (HA) in children. The objectives of our studies are to investigate differences in autonomic functioning between HA and normal children by means of peripheral indicators such as skin temperature, and to assess the effects of the drugs on both autonomic activity and on task performance which depends on attention such as reaction time. The hypothesis that HA and normal children respond differently to stimulant drugs has been tested in a study of the effects of d-amphetamine on autonomic activity and attention in 6 to 13-year-old normal and HA boys. On the same protocol male adults have been tested for age differences in drug effects. Studies have been done on the acute and chronic effects of caffeine on these measures in boys and in normal men.

PHS-6040
(Rev. 10-76)

Project Description

The general purpose of this project is to investigate the role of autonomic nervous system (ANS) activity and attention in hyperactivity in children and to study the effects of stimulant drugs on these processes in hyperactive and normal children and in normal adults.

Results of studies of d-amphetamine in normal and hyperactive children and in normal men have been described in previous annual reports and have been published (see also Z01-MH 00486-09 BPB). Briefly, the findings suggested similar behavioral and ANS effects of the drug in normal and hyperactive boys. In men, d-amphetamine produced many effects similar to those found in the boys, but at a high dose (.5 mg/kg), some differences were found. These can be conceptualized as a decrease in the specificity of ANS reactions to imperative or demanding stimuli and situations while in the boys there was an increase in this specificity. The ANS effects of amphetamine on men are remarkably similar to those obtained in comparing drug-free schizophrenics with controls (see Z01 MH 00484-21 (LPP) and suggest a common mechanism may be involved in both reactions.

Studies have been finished testing the effects of caffeine in boys and men using the same experimental procedures as were used in the amphetamine studies. The objectives of these studies is to compare the effects of these two "stimulant" drugs and, since caffeine has been reported to be a competitive inhibitor of the benzodiazapine receptor, to evaluate it as a possible pharmacological model of anxiety.

Results of two studies in boys were reported last year. An acute study using 3 mg/kg and 10 mg/kg found a partial concordance with expectations based on the literature on clinical anxiety. In a chronic study in which high and low caffeine consuming boys used caffeine or placebo daily for two week periods only very modest drug effects were found, suggesting development of tolerance. However, at baseline, high consumers showed low skin conductance reactivity compared to low consumers. It cannot be determined if this is an effect of chronic caffeine consumption, withdrawal, or a trait difference between high and low users.

The final study in this series tested the acute effects of caffeine on adult males who are high or low consumers. Results show caffeine increased skin conductance indicators of ANS arousal as it had in the boys. Heart rate (HR) showed a biphasic response: slowing at 3 mg/kg and speeding at 10 mg/kg. Boys also showed a biphasic HR response but had slower HR at both doses compared to placebo. In men caffeine reduced the tonic ANS response to the mild stress of task performance, an effect not as clearly evident for the boys. The increase in skin conductance responses after caffeine doses seen in the boys was not found in the men. However, like the boys in the chronic study, the high caffeine consuming men showed relatively low skin conductance responsivity to stimuli. Since this difference was independent

of caffeine dose, it cannot be attributed to withdrawal effects. Very few interactions between consumer status and caffeine effects were found suggesting that caffeine affects high and low habitual users in a similar manner.

These studies are significant for biomedical research and the program of the institute in several ways. First, the study of ANS changes after drug administration may help elucidate the mode of action of the clinical effects. For example, the more "focused" ANS activity in children and less focused ANS activity in adults produced by amphetamine can be seen as consistent with the improvement in attention seen in children and the adverse effects in adults from amphetamine abuse and the exacerbation of symptoms in schizophrenia by amphetamine. Second, since the pharmacological effects of these drugs are partially understood, these studies can elucidate the mechanisms of ANS activity and help to interpret the ANS findings on clinical populations. Third, amphetamine abuse is a public health problem and caffeine abuse may be one. Further understanding of the biological and psychological effects of these drugs may help in dealing with these problems.

Publications:

Zahn, T.P., Rapoport, J.L., and Thompson, C.L.: Autonomic effects of dextroamphetamine in normal men: Implications for hyperactivity and schizophrenia. Psychiatry Research, 4: 39-47, 1981.

Rapoport, J.L., Buchsbaum, M.S., Weingartner, H., Zahn, T.P., Ludlow, C., and Mikkelsen, E.J.: Dextroamphetamine: Cognitive and behavioral effects in normal and hyperactive boys and normal men. Arch. Gen. Psychiatry, 37: 933-943, 1980.

Elkins, R., Rapoport, J.L., Zahn,T.P., Buchsbaum, M.S., Weingartner, H., Kopin, I.J., Langer, D., and Johnson, C.: acute effects of caffeine in normal prepubertal boys. Am. J. Psychiatry, 138: 178-183,1981.

Rapoport, J.L., Elkins, R., Neims, A., Zahn, T.P., and Berg, C.J.: Behavioral and autonomic effects of caffeine in normal boys. Developmental Pharmacology, in press.

Elkins, R., Rapoport, J., Neims, A., and Zahn, T.: Behavioral effects of caffeine in normal boys. Nutrition in Health and Disease, in press.

Rapoport, J.L., Jensvold, M., Elkins, R., Buchsbaum, M.S., Weingartner, H., Ludlow, C., Zahn, T.P., and Neims, A.: Behavioral and cognitive effects of caffeine in boys and adult males. J. Nerv. Ment. Dis., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00488-16 LPP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Individual Differences in Survival and Reproduction Among Old Colony Mennonites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Gordon Allen	Guest Worker	LPP NIMH
OTHER:	Calvin W. Redekop	Professor of Sociology and Anthropology	University of Waterloo and Conrad Grebel College

COOPERATING UNITS (if any)

University of Waterloo and Conrad Grebel College

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Laboratory of Psychology and Psychopathology

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was undertaken to seek evidence of natural selection with respect to human behavioral characteristics. The population chosen consisted of about 13,000 German-speaking Mennonites whose culture resembles in many respects that of pre-industrial European peasants.

A census-survey was made in 1967 and vital records of the church were copied. For 569 families in the file, data include complete vital records and factor scores on several dimensions of social and economic behavior. Lengthy editing processes have now been completed and final analysis is in progress. This will first, specify parameters of survival and reproduction; second, explore interrelations among vital events such as effect of infant mortality on birth intervals; third, examine the effects on fertility of such variables as wealth, medical care, and social status.

PHS-6040
(Rev. 10-76)

Project Description:

This investigation was undertaken in 1965, mainly to seek evidence of natural selection with respect to human behavioral characteristics. The study of selection in a pre-industrial type of European culture may indicate some of the influences that have produced present genetic tendencies and would draw a base line for future studies of selection under modern conditions. A secondary objective was to define natural patterns of marriage and reproduction that might have a bearing on the growth and regulation of human populations.

The population under study consists of about 13,000 German-speaking Mennonites in a politically integrated cluster of 46 villages in northern Mexico. These people were selected because of their simple and uniform culture insulated from mass communication media and because of their apparently unrestricted fertility. Also important was the fact that, unlike some similar religious isolates, this society supports a considerable degree of economic competition.

Professor Calvin Redekop, of the Department of Sociology and Anthropology at Conrad Grebel College and the University of Waterloo, has assisted in the planning and execution of the study. He speaks the Low German dialect of these people and has lived among them.

Information was gathered by means of a combined census and survey that reached a third of the population. This was supplemented with photographs of all vital records kept by the church, a public list of farmers and their holdings, a list of all village and church leaders in the 40-year history of the population, and records of Mennonite births in local hospitals.

Data on births, marriages and deaths, including complete reproductive histories, were cross-edited between church and census records with the elimination of many errors and omissions in both sources. This editing was completed at the end of 1979. On 569 families, we now have complete vital records, the results of the socio-economic survey, and factor scores on several dimensions of social and economic behavior.

Analysis will be directed to at least the following three subjects:

1. Life table analysis of female fertility.
2. Effects on male and female fertility of age at marriage, infant deaths and widowhood/remarriage.
3. Correlation of fertility with socio-economic variables including wealth, use of medical technology and social status.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00489-24 LPP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Variables Affecting Twin Birth Frequencies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Gordon Allen Guest Worker LPP NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.0	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Significant variations in the twinning rate can be observed both in the long term and in the short term. Attempts to explain such variation have been based variously on coital frequency, declining sperm counts, a fertility advantage in twin-prone women, variation in early loss of embryos, and other phenomena.

Statistical findings in this project complement endocrinological research and implicate social and psychological influences on gonadotrophic hormone levels. Since the latter control estrogen and progesterone secretion, this phenomenon links social events to health and fertility in ways that will be explored further.

PHS-6040
(Rev. 10-76)

Project Description:

This project, begun in 1957, has had three phases. In the first, U.S. birth statistics were used to document variations in twinning rates over space and time. This led to new support for an old hypothesis; namely, that twin-prone women are more fertile than others. If true, this would explain much of the variation in the twinning rate. A quantitative estimate was obtained for the fertility advantage required of these women to explain the observations.

In the second phase, data were contributed by the Perinatal Research Branch, NINCDS, on 50,000 births studied prospectively. Analysis of these births, together with the complete reproductive histories of the mothers, permitted a rather decisive exclusion of the hypothesis of increased fertility in mothers of dizygotic twins.

The third phase sought to resolve the paradox presented by evidence for and against elevated fertility in twin-prone women. The emerging hypothesis has implications both for fertility control and for health.

Findings:

Some prominent features of the distribution of twin births can be explained nicely by the hypothesis that social events in women's lives, especially the making and breaking of close relations with men, affect gonadotrophin secretion. This hypothesis now appears very plausible in light of the recent finding by LaFerla that sexual arousal by erotic films raises women's gonadotrophin levels. The statistical and physiological findings complement each other and may lead to new understandings of reproductive physiology and health.

A relatively minor result of this study has been a fuller understanding of why the dizygotic twinning rate varies with age, parity, race, family and social circumstances, while the monozygotic twinning rate remains extraordinarily constant. Monozygotic twinning occurs in an early embryo that has established the stable conditions necessary for normal development; division of the embryo appears to be an uncontrollable, truly random event, possibly depending on cell numbers or molecule numbers. Double ovulation, in contrast, occurs at the organ or organism level, subject to innumerable physiological and now, psychological effects, and the two embryos have nearly independent chances, instead of highly correlated chances, of survival.

Publications:

Allen, G. The twinning and fertility paradox. Proceedings of the Third International Congress on Twin Studies. New York: Alan R. Liss. In press.

Allen, G. Errors of Weinberg's difference method. Same proceedings.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00491-05 LPP

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Personality factors and psychophysiological responses to changing stimulus input.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Theodore P. Zahn	Research Psychologist	LPP	NIMH
Other:	Thomas N. Robinson, Jr.	Guest Worker	LPP	NIMH

COOPERATING UNITS (if any)

None

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.5	.5	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to investigate relationships between differences in personality, sensory thresholds, and autonomic nervous system (ANS) activity in normal humans and to study racial differences in ANS activity. Bilateral skin conductance, heart rate, vasomotor activity and respiration have been recorded in two sessions in which constant and variable intensity tones and lights are presented and auditory and two flash thresholds determined by methods which permit signal detection analyses. Several standardized personality tests were also given. The procedures allow determination of the effects of stimulus intensity and heteromodal stimulation on ANS activity. The protocol allows testing of several theoretical models of the relationships of ANS activity, sensory sensitivity and personality, some of which have implications for the etiology of psychopathology.

PHS-6040
(Rev. 10-76)

Project Description

A large body of psychological literature postulates that an important dimension of individual differences in behavior or personality is reflected in the reactions of the nervous system to sensory stimulation. Pavlov's original conception of "strong" and "weak" nervous types has been modified and extended by Western theorists to reflect such personality dimensions as "extraversion-introversion", "sensation seeking", and "field dependence", each of which can be measured by a questionnaire or other test procedures. The theoretical models that have been built up from these concepts have implications for interrelationships between personality, autonomic nervous system (ANS) base levels and responsivity to stimulation, and sensory sensitivity. There are also implications for psychopathology in that schizophrenics have been considered to be extremely "weak" nervous types in the Pavlovian system (i.e., overreactive to weak stimulation and underreactive to strong stimulation--"transmarginal inhibition"). Another development is the more recent delineation by H. Eysenck of the dimension of "psychoticism."

The major objective of this project is to test some of the implications of these models of personality by interrelating the personality measures with sensory thresholds and sensitivity, and ANS activity in normal humans. Other objectives are to assess racial differences in ANS activity and in its relationships to the other variables in the study and to explore relationships of differences in the laterality of skin conductance activity with behavioral assessments of laterality.

Over 180 normal volunteers have been assessed on several personality dimensions including the Eysenck scale of extraversion, neuroticism and psychoticism, field dependence, sensation seeking, impulsivity, ego strength, and anxiety, assessed for degree of lateral dominance and given tests of ANS and sensory functioning in two separate sessions as described earlier.

Results to date show that contrary to the prediction that introverts should show a transmarginal inhibition introverts compared to extraverts had large ANS reactions to high intensity auditory stimulation. Nor do the predictions of these theories with respect to the interrelationships between personality, ANS "arousal", and sensory thresholds hold up since we found that subjects giving evidence of higher ANS "arousal" showed high thresholds and low sensitivity and subjects who, from the personality measures would be expected to have "strong" nervous systems showed greater sensory sensivity. However, a limited confirmation of the predictive validity of the "psychototicism" measure was found. Subjects scoring high and low on this scale differed in the direction of the relationships between ANS arousal and two-flash threshold and sensitivity. These are similar to results found in comparing schizophrenic and normal subjects.

Few previous studies of laterality of electrodermal functioning have compared right and left handed subjects. Preliminary analyses of our data show that left handed subjects show greater left hand electrodermal

responsivity under all conditions while right handed subjects show greater right hand responsivity during threshold determinations and in instruction and rest periods but greater left hand responsivity while listening to nonsignal tones. This supports the general hypothesis that the cerebral hemispheres in right and left handed subjects do not function simply as mirror images of one another.

Analysis of this large data base is continuing. In addition to further tests of personality theories and laterality differences, racial differences will be studied.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00495-05 LPP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
The Psychobiology of cognitive processes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	Michael H. Ebert	Chief, Sec. Exp. Therapeutics	LCS	NIMH
	J. Christian Gillin	Research Psychiatrist	BPB	NIMH
	Philip Gold	Chief, Unit on Neuroendocrin.	CPB	NIMH
	Harry Keiser	Clinical Director	HEB	NIMH
	Dennis Murphy	Chief, Neuropharmacology Br.	CNB	NIMH
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	Robert Post	Chief, Sec. on Psychobiology	BPB	NIMH
	Judith Rapoport	Chief, Unit on Child. Ment. Illness	BPB	NIMH
	Stanley I. Rapoport	Chief, Lab. Neurosciences	LN	NIA
	Edward Silberman	Staff Psychiatrist	LPP	NIMH
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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are investigating the psychobiology of cognition in man. We attempt to interrelate psychological and biological determinants of various components of cognition such as the specific and discrete psychobiological mechanisms that define the acquisition, processing, consolidation and retrieval of experience. Experiments are designed to examine the biological and psychological determinants of psychiatric and neuropsychiatric alterations in cognitive processes in adults and children. These same cognitive processes are examined under drug altered conditions. Some of these studies have examined whether drugs that enhance aspects of cognition would reverse cognitive disturbances in patients. Findings include changes in encoding, memory and retrieval that occur in response to drug treatments that alter cholinergic, noradrenergic and neuroendocrine central nervous system activity, as well as cognitive changes produced by neuropeptides, in the dementias, aging, and in depressed patients. All of the studies are designed to help define the psychobiology and determinants of cognition by continuing forms of cognitive changes that are apparent in a variety of syndromes and in response to changes in central nervous system activity.

Project Description:

The research projects reviewed here are all concerned with the psychobiology of cognitive processes in man. They have been designed to explore the psychological and biological determinants of various aspects of cognitive processes and their interrelationships. Studies have examined the psychobiological processes that define the processing, learning, and storage of information, new experience, how processed events are altered, or elaborated in memory, the consolidation and retention of information, and the mechanisms that are involved in retrieval of stored information. Some of the components of cognitive processes that have been examined include attentional determinants, aspects of short-term memory, the consolidation of information in long-term memory, the kinds of state or trait specific cognitive strategies, "effortful" vs. automatic cognitive processes, and the kinds of strategies that subjects use to retrieve experience that they have stored in memory.

Two broadly conceived strategies are used to define the psychobiology of cognitive processes. One is to contrast the effects of different treatments on different components of cognition, such as on attention, information storage, consolidation, encoding, (automatic and effortful) and retrieval processes. These studies include: 1) pharmacological manipulations, such as cholinergic drugs, noradrenergic drugs, abused drugs (alcohol, marijuana), central nervous system depressants, neuropeptides, and 2) behavioral manipulations that alter arousal/activation, stimulus attributes (altering encodability) and types of stimulus processing strategies subjects use to process information. These studies are carried out in normal persons, as well as in patient groups with different forms of psychopathology. Would different kinds of pharmacological or behavioral manipulations of cognition lead to different forms of enhanced or disrupted cognition? Contrasting manipulations of different systems in the CNS producing different cognitive effects would help provide a picture of the structure of the psychobiology of cognition. The rationale for the alternative strategy for researching the psychobiology of cognition is to contrast the different forms of cognitive failures seen in different psychiatric and neurological syndromes. Would the disrupted cognition seen in some psychopathological states be qualitatively and quantitatively different and related to specific changes in central nervous system activity? For example, how are the amnesic/cognitive impairments seen in Huntington's disease, Korsakoff's syndrome, and Alzheimer's disorder different? How might the differences be an expression of the specificity of central nervous system involvement in each of these disorders? In some instances, the possibility of discriminating between the form of the cognitive impairment is necessary for both adequate diagnosis and effective treatment, i.e., such as the cognitive disruptions that are part of depression as opposed to that produced by a progressive dementia. Frequently, depression is an integral part of a progressive dementia, and the cognitive impairment is a joint product of the two disorders. Some studies have also investigated the therapeutic potential of various psychoactive drugs and behavioral treatments. Do these attenuate or reverse the cognitive disruptions seen in various forms of dementia, hyperactivity and learning disability syndromes in

children, depression, mania and the schizophrenias? Each of the studies is clinically relevant, as well as pertinent to understanding unimpaired cognitive processes.

In summary, all of the parts of this project are concerned with defining the discrete psychobiological components of cognitive processes that are involved in the appreciation, storage, retention, and retrieval of experience. The studies are concerned both with the bases of cognitive processes as well as clinical studies of disordered cognition.

1. Pharmacological alterations (enhancement and disruption) of cognitive processes

These studies have contrasted the (cognition) enhancing effects of cholinergic agents such as arecoline, physostigmine, THA, and lecithin treatment with those of amphetamine and neuropeptides in both unimpaired subjects, as well as in patients (hyperactive children, Alzheimer's disorder, depressed patients). Would drug treatments that lead to qualitatively different central nervous system effects produce systematically different changes in cognitive processing? That is, would enhancements or disruptions of cognition occur through different psychobiological mechanisms, and might the cognitive response to different drugs make such a pattern discernible? Some neurotransmitter antagonists have also been used to model forms of impaired cognition in man.

In some instances, drugs that might disrupt aspects of the acquisition of information, may enhance some other stage of cognitive processing (e.g., process-specific effects of drugs that alter cognition can be used to better define a psychobiology of information processing in man. This research has also involved attempts to find clinically useful strategies for altering disrupted cognition in man.

2. State-dependent learning

This area of research provides a useful framework for exploring: a) the qualitatively unique manner in which events are stored (encoded and retrieved from memory), b) studies of disturbances in mood state and how these define mood specific strategies for processing experience and remembering past events in memory, c) qualitative changes in cognition in response to psychoactive drugs, d) contextual factors as determinants for defining the nature of trace events in memory, e) individual differences in susceptibility to state dependent or dissociative mood/drug effects.

3. Memory Consolidation

This research has focused on the psychobiological events that follow the acquisition (storage) of information and which occur well before processed information is to be retrieved from memory. Studies in both normal subjects and patients have examined the form and strength of stored information in memory and the processes that might further sustain, enhance, or disrupt

stored trace events that are already part of memory. The rate of decay of information, the susceptibility of information to interference and biological factors, may be important determinants in defining what is available and accessible in recall, once information has been stored in memory. Disruptions in consolidation may contribute to the cognitive failures in the dementias.

Drugs that disrupt memory and learning may do so by altering biological operations that succeed acquisition or learning. Likewise, drugs, e.g., neuropeptides may enhance aspects of learning and memory by facilitating the consolidation of learned information.

4. Behaviorally defined mechanisms that alter components of cognition

Characteristics of stimuli such as a) organizational properties, b) imagery, c) emotional arousing attributes of stimuli, d) information presentation rate, e) mode of processing, f) language vs. pattern information, g) types of learning have been studied in relation to its effects on attention, acquisition (learning), strength of learning, retention, and components of information retrieval. The studies have examined these factors in normal controls, as well as in patient groups (depressed patients, hyperactive children, learning disabled, patients suffering from various forms of dementia). Some of the issues raised in these studies include the following: How might aspects of information processing alter the attentional, short-term memory, encodability, retention, and retrieval of information? Would different forms of psychological manipulations systematically alter different aspects or components of cognitive processes? Do patients who demonstrate failures to learn and remember do so because of disruptions in some, but not all of these component cognitive processes, and can manipulations of some characteristics of information processing change these disruptions in cognition? Recent studies have also begun to explore "effortful" vs. "automatic" information processing and how these are altered under different motivation/arousal conditions. This approach to cognition has been used to define the effects of depression on cognition, determinants of cognitive failures in the learning-disabled child, and drug-altered changes in cognition (see below).

5. Cognition and mood

Studies have included research of mood-related changes in: 1) the brain lateralization of cognitive functions; 2) arousal and activation and its role in information processing; and 3) the encoding and retrieval of events in normal mood and in depression or mania. This research has examined how patients with disturbances in mood process information in a mood-state specific manner. In addition, this research has begun to examine mood-related changes following psychoactive drug treatment and its interactive role in altering cognitive processes. Other research has explored the degree to which effortful processing of information is compromised as a motivation-related determinant of thinking in depressed patients.

6. Mechanisms of cognitive impairments that determine forms of learning disabilities

Studies have been designed which investigate a) forms and incidence of various kinds of learning-disabilities in children, b) the descriptive nature of the learning disabilities in these children; c) potential strategies for their remediation.

Methods

Three strategies have been used in these studies. One involves manipulation of different biological systems that may play a role in different aspects of cognition in man. Various neurotransmitter agonists and antagonists, as well as agents that effect neuroendocrine functioning are contrasted to one another in both impaired and unimpaired subjects. A second strategy involves systematic comparison of various forms of cognitive failures apparent in different clinical groups. These methods use neuropathological, neurochemical and neuroanatomical changes that are apparent in different forms of chemical syndromes to provide a matrix of the biological determinants of these different forms of impaired cognition. The third set of methods involves systematic manipulation of stimulus, retention processing, and retrieval conditions.

A large variety of cognitive strategies have been designed to explore the psychobiological components of cognition in the studies that are part of this project. These strategies have included modified forms of current methods used in human information processing research, as well as newly-developed tools that might more adequately examine determinants of cognition in clinical studies and those assessing cognitive drug effects. These strategies have been developed in a number of studies and include measures and manipulations of: a) organization of information, b) informational context for processing information; c) stimulus attributes such as imagery, emotional properties, frequency, d) forms of learning and recall (free recall, prompted free recall, recognition memory, cued recall, serial learning, paired associates learning); e) processing time and type of presentation of information; f) type of processing strategy (processing on the basis of meaning or sound properties); g) immediate vs. delayed recall of information with or without rehearsal of stored information; h) presentation of language vs. pattern information to left vs. right hemisphere (methods used to investigate lateralization); i) rapid (tachistoscopic) presentation of information; j) measurement and manipulation of different forms of retrieval of information in memory, including forms of free recall, prompted or cued recall, recognition memory, method of "savings;" k) very long-term memory retrieval; l) assessment of effortful and automatic cognitive processes; m) arousal and motivation in information processing.

During the past year the studies of disordered cognition that result from being in a depressed mood state have allowed us to define, with some precision, the kinds of cognitive processes that are impaired as well as those that are left relatively intact in patients with disturbances in mood

(depression). We have noted that while depressed patients demonstrate impairments in aspects of learning, memory, and retrieval as a function of the intensity of their depression, retrieval of experience is, in part, mood-state dependent. Some aspects of information processing are particularly likely to be interrupted in these patients. Using techniques that distinguish between automatic versus effortful cognitive processes (passive versus active processing) in cognition we have noted that depressed patients are impaired cognitively to the extent to which cognitive processes or operations require effortful rather than passive or automatic kinds of operations. The depressed patient who may demonstrate profound impairments in learning and memory is indistinguishable from a normal control automatic processing tasks. In addition, the extent to which these depressed patients are able to manifest effort is highly correlated with the intensity of their depression and likewise is highly correlated with the extent to which they can perform these non-automatic active cognitive operations (Cohen and Weingartner, Archives of General Psychiatry, in press.

It is also clear that the extent to which depressed patients are provided with organization or structure in an information processing task is the extent to which they are relatively indistinguishable from normal controls in terms of learning memory functions. When processing random information depressed patients demonstrate profound cognitive deficits. On structured tasks these deficits are attenuated (Weingartner, et al. Archives of General Psychiatry, 1981). The last finding has been particularly important in distinguishing between the cognitive dysfunction of depression and progressive dementia.

The most striking clinical manifestation is in patients with progressive idiopathic dementias. Recent findings from our laboratory have now defined some of the characteristics and determinants of the cognitive dysfunction in these patients. We know that information is relatively rapidly lost from memory, immediate memory is relatively unimpaired, and any type of learning-memory operation that requires the establishment of permanent trace events in memory is dramatically disrupted. Thus failures are, in large part, due to processing or acquisition deficits which then result in weak trace formation and therefore failures to retain information in memory. A considerable body of research has suggested a distinction between semantic memory and the depository of information of knowledge structures from episodic memory, i.e. memory for ongoing recent events. Although these two kinds of memories have been traditionally viewed as being separate and distinct we have found an important link between the two. Based on recent findings relating these two systems it has been possible to account for many aspects of the memory impairment in progressive dementia patients. In a series of studies we have been able to demonstrate that the extent to which Alzheimer's patients have access to structures in semantic memory is the extent to which they are relatively unimpaired on many tasks of learning and memory. These results have important implications both diagnostically in distinguishing this group of cognitively impaired patients from other groups (eg., cognitively impaired depressed patients) as well as for potential treatment strategies that would be useful for these patients (Weingartner et al., Journal of Abnormal Psychology, 1981.

The findings have suggested that cognitive failures in progressive dementia are distinguishable from those evident in depression, and other syndromes. This has prompted active study of drug and other treatment strategies for reversing such cognitive failures. By understanding both the mechanisms of cognitive impairments and the neurochemical response following various forms of drug treatment, it should be possible to systematically design studies that would effectively examine the therapeutic potential of cholinergic agonist drugs that would stimulate catecholamine activity. We also have used neuropeptides such as vasopressin. These studies have been initiated during the past year. The mechanisms and determinants of the cognitive impairments in depression and dementia have allowed us to devise strategies to distinguish clinically between these two groups. Characteristics of automatic versus effortful processing, the extent to which effort is extended in accomplishing tasks and the processing of unrelated vs. related events allows us to distinguish clinically between the cognitive impairment in depression and that in the progressive idiopathic dementia patients.

In a series of studies we have attempted to describe the components of cognitive changes that are apparent in children with various forms of learning disability. We have examined two groups of these children, one where hyperactivity as part of the syndrome, and a second group where there is no evidence of hyperactivity or generalized retardation. Nevertheless, these children demonstrate dramatic impairments in learning and memory that resemble the kinds of disruptions in cognition that are evident in some groups of adults. The resemblance is closest to depressed patients; it also resembles the kinds of cognitive changes that are produced by drugs that disrupt cholinergic and noradrenergic activity. Both hyperactive and learning disabled children show impairments in effortful processing of information; automatic processing is left relatively intact. On incidental learning paradigms these children are indistinguishable from normal controls. Both groups of children also demonstrate impairments in those characteristics of cognition that require the imposition of organization in memory. In many ways the results we have obtained to date would suggest that the type of cognitive impairment seen in these children resemble those seen in depressed patients in contrast to the pattern of cognitive impairments evident in progressive dementia patients. The kinds of cognitive impairments are also like those that are apparent when unimpaired subjects are treated with drugs that disrupt or block catecholamine activity. These results are presently being prepared for publication including a review article which will be submitted to the Psychological Bulletin.

Pharmacological Studies of Cognition in Man

We have been able to demonstrate both in patient groups as well as in unimpaired subjects that the effects of cholinergic antagonists and agonists produce cognitive changes that are qualitatively different from those of drugs that have their major effect on catecholamine activity, either as agonists or as antagonists. We have also completed a series of studies demonstrating the role of neuropeptides such as synthetic vasopressin-like substances in terms of their role in enhancing aspects of learning and memory in both cognitively impaired depressed patients (Weingartner, et al., Lancet, 1979)

in unimpaired subjects and in patients with progressive idiopathic dementia (Weingartner, et al., submitted to Life Science, 1981). The general pattern of these findings implies that different neurotransmitter systems and different kinds of neurochemical mediators are involved in the regulation of various aspects of the acquisition, retention and retrieval of information.

Major Findings: Studies of psychobiological determinants of cognition in impaired subjects

We have demonstrated that cholinergic mechanisms play a role in aspects of information acquisition and in the storage and retrieval of information. Scopolamine treatments which disrupt cholinergic activity produce an impairment in information processing. This scopolamine-induced impairment in the acquisition of new learning can be reversed by arecoline treatment. The scopolamine-induced disruption in cognition appears to model, in normal subjects, many of the characteristics of cognition seen in untreated patients with Huntington's disease.

In another study, it was possible to show that cholinergic mechanisms may also be involved in the consolidation of information in memory. When subjects learn information in an undrugged state, and afterwards are treated with arecoline, there is a comparable facilitation of information later recalled, when compared to learning and recall that occurs under arecoline treatment conditions.

Amphetamine treatment also increases the amount of information which can be recalled following various modes of input processing under drug state conditions. Unlike cholinergic manipulations, amphetamine appears to amplify or strengthen trace events in memory rather than increasing the total amount of learning (size of the pool of trace events in memory). In a series of studies, it has been possible to show that amphetamine produces an enhancement of some components of cognition in depressed patients, hyperactive children, normal children, and normal adults. Amphetamine also induces a change in state which serves as a state-specific context biasing how information is interpreted and remembered. Amphetamine treatment, like cholinergic treatment, produces state-dependent retrieval. The contrasting enhancing effects of cholinergic agents and amphetamine, and the cognitive disrupting effects produced by scopolamine vs. lithium has served as one strategy for exploring the specific psychobiological mechanisms that may define different components of cognitive processes.

Alcohol, when administered after the processing of information, produces an enhancement in recall when tested in the unintoxicated state. Most recent findings suggest that alcohol induces a brief excitatory phase (possibly mediated by changes in catecholamine activity) which affects memory consolidation. This excitatory phase may be important in defining some of the reinforcement properties of alcohol. This paradoxical cognitive facilitating effect of alcohol, administered during a consolidation phase of memory, highlights the differentiated mechanisms and components that make up information processing, memory, learning, and retrieval.

We have now completed a series of cholinergic trials in Alzheimer's patients and have demonstrated that cholinergic antagonists such as scopolamine mimic many of the characteristics that are evident in progressive idiopathic dementia, (Weingartner, et al., in preparation for Memory and Cognition). We have also noted that combinations of cholinergic agents do in fact produce small but reliable enhancements of some aspects of learning and memory in patients with Alzheimer's disease. The limiting factor here has been that the extent to which an enhancement in learning and memory is evident is largely a function of the degree of cognitive intactness of the patients (Kaye, Sitaram, Weingartner, Lancet, submitted for publication; Kaye, Weingartner, et al., ISG.)

While alcohol has been viewed traditionally as one type of pharmacolgical manipulation that reliably produces learning and memory impairments in man, recent work from our laboratory in collaboration with NIAAA has demonstrated that post-processing manipulation including treatment with alcohol can in fact produce some enhancements in learning and memory. The focus on the biological and psychological events that follow the initial acquisition of information has generally been ignored in studies of cognitive processes. This consolidation phase of memory, and the biological events that occur during this time may be important in establishing permanent records of experience. This is evident both in our studies using alcohol, with the paradoxical enhancement of what has been stored in memory, and in the effects of vasopressin on reversing retrograde amnesia following ECT administration. It would be important to continue to explore in greater detail biological events that succeed information processing in man (Weingartner, et al., Science, 1981; Parker, et al., Psychopharmacology).

Amphetamine drug trials of state-dependent learning in children have demonstrated striking individual differences in dissociative effects. Fifteen children have been studied under four different experimental conditions including: a) learn under drug condition (LD), recall under drug condition (RD); b) learn following placebo treatment (LP), recall following placebo treatment (RP), (these are congruent learning-recall conditions); c) LD, RP; and d) LP, RD, both of which are disparate learning-recall conditions. Somewhat more than half of these children show clear dissociative effects: when learning occurs under one condition, e.g., following amphetamine treatment, but memory is tested in the undrugged state, then memory appears to "fail," particularly for poorly processed items, or those that are recalled without benefit of cues or prompts. These findings point to individual differences in the amphetamine response with respect to the form of the cognitive effects in hyperactive children. These dissociative effects also have clear and important implications for the classroom functioning of stimulant-drug-treated hyperactive children.

Summary

Ongoing studies have yielded findings which permit us to examine how a) specific aspects of cognition are altered by drugs that affect activity in different parts of the central nervous systems, b) how different forms of

neuropathology and psychopathology lead to different alterations in the cognitive processes that normally would lead to successful appreciation, acquisition and retrieval of experience. The extensive findings from the studies described here suggest that distinctive psychobiological mechanisms are involved in the organization, encoding, consistent retrieval, and consolidation of information in memory. Different behavioral-neurochemical determinants may alter the strength of trace events in memory, their accessibility, and the amount of stored experience in memory.

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Weingartner, H., Kaye, W.H., Smallberg, S., Cohen, R.A., Ebert, M.E., and Gillin, C.: Memory failures: Distinctive patterns of cognitive dysfunctions in dementia and depression. Pharmacology of Memory Disorders. In press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00500-02 LPP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Cognitive and Perceptual Changes in Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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3.0	2.0	1.0

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to investigate the cognitive and perceptual changes which are present in, and characteristic of, major affective illness. The present investigation comprises nine separate ongoing studies: (1) Psychomotor and psychosensory symptoms in affective illness, patients with complex partial seizures, and patient controls; (2) perception and recall of emotional and neutral stimuli in depression; (3) hypothesis testing in depression; (4) Luria neuropsychological evaluation of depression, schizophrenia, and Alzheimer's disease; (5) cognitive and mood response to epinephrine in normal and depressed subjects; (6) lateralized hemispheric function in depression; (7) Halstead category test performance in depression; (8) visual and olfactory perceptual changes in depression; (9) relationship of cognitive dysfunction to diagnostic sub-type, and neuroendocrine abnormalities in depression.

PHS-6040
(Rev. 10-76)

Project Description

The premise of this project is that there are, in addition to the well-known mood changes, important alterations in perceptual and cognitive processes in major affective illness. Such symptomatology may have important implications for the pathophysiology of affective disease and may provide significant contributions to delineating clinically and prognostically homogeneous subtypes of this condition. The present study is composed of eight investigations, looking at various aspects of the problem. They are aimed at documenting the nature and extent of cognitive and perceptual changes at the clinical level, and of investigating the degree and structure of such deficits in the laboratory. Below is a summary of the current studies within this project.

1. Psychomotor and psychosensory symptoms in affective illness. A wide range of behavioral and perceptual manifestations, many with localizing significance in the brain, have been described as concomitants of complex partial seizures. The literature and clinical observation suggest that there may be areas of overlap between these phenomena and symptoms of major affective illness. The purpose of the present study is to investigate the nature and degree of such overlap. In collaboration with Dr. Robert Post, a structured interview has been devised to elicit presence of symptoms related to perception, thought processes, orientation, memory, and involuntary motor behavior. The interview is based on a survey of the literature describing such changes in epilepsy. Design of the project involves administering the interview to 120 patients; 40 are patients with a history of major affective illness currently being treated by units of the Biological Psychiatry Branch; 40 are patients with a firm diagnosis of complex partial seizures, under the care of the Epilepsy Branch, NINCDS (in collaboration with Dr. Roger Porter); and 40 are patients of the Hypertension-Endocrine Branch, NHLBI, screened for absence of psychiatric or neurological disease. At the present time, approximately 70 affective and epileptic patients have been interviewed. Reliability studies for the interview are now under way. On the basis of preliminary data, it appears that the two groups are similar in incidence of psychosensory symptomatology; it forms a prominent part of the clinical picture in roughly 25% of both groups. We are now beginning to interview the control group. Data will be analyzed to evaluate the relationship of symptoms to course of illness, drug response, and personality characteristics of the subjects.

2. Perception and recall of emotional and neutral stimuli in depression. It is a common clinical observation that affectively ill patients often seem highly insensitive to their own internal emotional state. It is also well-known that depressed subjects perform more poorly than controls in a variety of memory tasks. The purpose of this study is to look systematically at how depressed subjects evaluate the emotional qualities of verbal material, how such evaluations change with changes in clinical status and how they interact with the subject's ability to recall the material. Fifteen depressed subjects were given a list of forty words to rate for

degree of emotionality on a zero to seven scale. Half the words were high emotion words, and half low emotion words, as determined by previous studies in normals. Subjects were asked to freely remember the words after they had been rated, and later to pick out the words from a list in which they are intermixed with distractors. Fifteen normal control subjects performed the identical task. In addition, whenever possible, subjects who had changed clinical state were again given the list for re-rating. To date, the collection of data is complete and the results are being analyzed. Normal and depressed subjects rate words similarly as to high or low emotionality, although depressed subjects' ratings of the high emotion words tended to be lower, with greater variance than normals. Depressed subjects remember fewer words, both freely, and on recognition, than normals. However, while in the normals there is no relation between emotionality ratings and memory performance, in the depressed group, the lower the ratings the fewer words were recalled. Furthermore, the depressed group showed evidence of preferential storage and retrieval of high emotion words, whereas normals show only preferential retrieval of these words. In the light of previous memory studies with normal and depressed subjects, this data suggests that the depressed group processes stimuli more shallowly than normals, and that sensitivity to emotional qualities of words is an indicator of ability to process deeply in the depressed, but not in the normal group. That the two groups classified high and low emotion words similarly suggest that the cognitive differences are not at the attentional level, but at a later stage of processing.

3. Hypothesis testing in depression.
Most cognitive research in depression has focused on memory-related impairments. The present investigation uses a learning paradigm which places relatively light memory demands on the subject, focusing instead on hypothesis formulation and testing. The task, devised by Levine, is a variation on the Wisconsin Card Sort. Subjects are presented with the Levine task, which is then repeated with our own modifications. The task involves a deck of cards with two stimuli on each card. Each stimulus has four attributes (such as color and size); the subject's task is to guess which attribute has been arbitrarily designated as "correct." Subjects are asked to point to the stimulus that they think has the chosen attribute and are given yes or no feedback on selected cards. The procedure is devised so that each administration can be scored for number of hypotheses formed, number of correct hypotheses, ability of the subject to narrow down his choices as more feedback is given, and degree of hypothesis changing or keeping after positive or negative feedback. In addition, our modification of the procedure allows discrimination between poor performance due to memory deficits, or to subjects' inability to formulate an appropriate strategy. Data collection is currently under way. Preliminary results indicate that depressed subjects are unable to focus on a correct hypothesis as well as normals. That this is not due to inattention is suggested by the fact that they form as many coherent hypotheses as normals. Our current evidence also suggests that the deficit is not primarily one of memory on this task, since forcing subjects to adopt an appropriate strategy (without giving them any

memory aids), significantly improves performance. Thus, depressed subjects appear deficient in ability to form useful problem-solving strategies. Such a deficit may relate to clinical phenomena such as depressed patients' overgeneralized thinking, and feelings of being overwhelmed by problems.

4. Luria neuropsychological investigation in depressed, schizophrenic, and Alzheimer's patients.

We are currently surveying depressed, schizophrenic, and Alzheimer's patients for deficits in higher cortical functioning, using the standardized Luria examination. The examination is based on the work of the Russian neuropsychiatrist, A. R. Luria, as standardized by Golden and his associates. The battery is divided into 11 sections based on various higher cortical functions, such as motor, tactile, speech, reading, and abstraction. There are also experimental scales assessing overall level of brain pathology and localization of pathology by hemisphere and lobe. It is hoped that the battery will provide indications for future investigations of specific functions of anatomic areas in these patient groups. Indications to date suggest left temporoparietal dysfunction in the schizophrenic group, and global deficits, including visual perceptual dysfunction in the Alzheimer's. Data in depression are not yet sufficient to provide reliable indications. We are planning to compare Luria results with presence and type of brain abnormality as shown by C-T scan in all three groups. Intergroup comparisons will also examine possible distinct cognitive profiles in demented and psychiatrically-impaired patient groups.

5. Cognitive and mood response to epinephrine in normal and depressed subjects.

This study has three major objectives. The first is to investigate the relationship between epinephrine-induced peripheral sympathetic arousal and performance on a verbal memory task in normal subjects. Effects on learning will be related both to pulse and blood pressure changes and to subjective mood and activation status of the subjects, as induced by the drug. The second objective is to compare the effects of adrenalin on physiologic and psychologic factors in normal and depressed subjects. Although schizophrenic subjects have been shown to have blunted physiologic responses to epinephrine, the comparable study in major depression has not been done. It has also been shown that autonomic arousal and cognitive set combine to produce a subjective mood state in normal subjects. Since depressed populations are known to have altered cognitive processes and appear clinically to show deficits in "emotional proprioception," mood response to adrenalin in this group is of theoretical interest. The third objective of the study is to investigate the ability of adrenalin, a peripherally acting drug, to induce state-dependent learning effects in normal and depressed populations. Drug-induced-state-dependent learning has been demonstrated for a variety of centrally acting drugs, but never with ones whose action is essentially peripheral. The study will also provide insight into the conditions necessary for the production of a dissociative state, in particular, the relationship between state-dependent phenomena and physiologic, subjective, and behavioral changes induced in the subjects. All phases of the project involve intravenous infusions of the drug at a carefully controlled rate, with continuous cardiac and blood

pressure monitoring. The initial phase of the study, involving normal volunteers only, has recently gotten underway. Early data suggest changes in learning and memory, level of activation, and anxiety level, but not other aspects of mood under the influence of the drug.

6. Lateralized hemispheric function in depression.
There are presently two distinct parts to this study. The first involves an extensive review of the literature concerning hemispheric lateralization of mood-related behavior and emotional aspects of cognitive processing. Taken as a whole, the current literature suggests that while the right hemisphere appears predominant in the control of many aspects of emotional behavior and processing, there is also a division of function between the hemispheres, with the right hemisphere dominant for negative affects and the left dominant for positive affects. Evidence for these two types of lateralization comes from a wide variety of experimental paradigms and subject populations, each providing a relatively small number of studies. It is clear from the literature that a great deal of confirmatory work must be done in normal, brain-lesioned, and affectively ill populations before the relationship of laterality to mood state can be understood. The literature review is currently being prepared for publication.

The second part of this study involves lateralized tachistoscopic presentation of visual material to depressed and control subjects. The task involves the subject making "same" and "different" judgments on material that can be processed either linguistically (left hemisphere) or on the basis of form (right hemisphere). Differential performance of the two hemispheres will be compared in the two subject groups. This part of the study is currently in the final stages of technical adjustment, and data collection will be initiated soon.

7. Halstead category test performance in depressed subjects.
Dr. Robert Savard of the Clinical Center Social Work Department has been investigating the performance of depressed subjects and matched controls on the Halstead Category Test, a portion of the Halstead-Reitan neuropsychological battery. Data based on the total test scores indicate that depressed subjects, particularly bipolars, and particularly older subjects, produce error rates comparable to patients with organic brain deficits. In this phase of the investigation, two questions are being examined. The first is whether improved performance of depressed patients in the recovered state is due solely to the state change, or whether they are also able to learn while depressed. Current data indicate that the learning curve for depressed subjects is considerably more shallow than for controls. The second question involves difference between patients and controls on the seven subjects of the Category Test. A significant groups x subtests interaction is present between the two groups. This appears to be due to proportionally worse performance by depressives on those subtests most heavily dependent upon spatial aspects of the stimuli. This finding may be suggestive of right hemisphere processing deficits in this group.

8. Visual and olfactory perceptual changes in depression.
This study was initiated in order to investigate whether cognitive impairment found in depression might, in part, be due to deficits at the level of elementary sensory processing. Because of the intimate relationship of the olfactory system to limbic areas of the brain that are involved in regulation of emotions, the sense of smell was chosen for the initial phase of the investigation. Threshold detection level, and psychophysical judgments of intensity and pleasantness were obtained from three groups of subjects: depressed, hypomanic, and recovered patients, temporal lobe epileptics, and normal controls. Data on 27 patients, 15 epileptics and 12 controls has been collected. There are no differences in detection levels of four odorants, two pleasant and two unpleasant, among the three groups. In their ratings of pleasantness and intensity, patients in all three states performed like normal controls. Epileptics, however, showed supersensitivity to odor intensity and pleasantness in their ratings. This difference appears to occur at a later stage of evaluation rather than at the primary stage of olfactory sensitivity. To investigate the possibility of subtle changes in the depressed group that might have been missed, we are preparing a psychophysical scaling task for two olfactory and two visual stimuli. On the basis of this task, sensitivity and response biases of normal and depressed subjects can be carefully quantified and compared.

9. Relationship of cognitive deficit to diagnostic sub-type and neuroendocrine changes in depression.
The purposes of this study are to attempt to examine cognitive dysfunction in depression as a function of of diagnostic sub-type. The study is being run in collaboration with the Evaluation Unit at the Psychiatric Institute, Washington, D.C., headed by Dr. Steven Targum. Classification procedures for depressed subjects include complete DSM III diagnosis as well as biological indices provided by the dexamathasone suppression test and the TSH response to thyrotropin releasing hormone (TRH). Cognitive testing is designed to examine not only level of performance, but the structure of deficits. A battery of six memory tests have been prepared for this study. The tests assess the effect of type of processing (deep vs. shallow), type of recall (free vs. cued), type of stimuli (high vs. low emotional, high vs. low imageable), and level of organization of the stimuli. Level and structure of cognitive performance will be examined as a function of diagnosis, and also in relation to underlying metabolic abnormalities.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00501-02 LPP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pharmacologic Studies in Depression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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1.0	.75	.25

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project covers studies examining behavioral and biological response of depressed patients to pharmacologic agents. Three separate studies are involved. The first is an extension of a previous study examining clinical response of depressed subjects to amphetamine; we are now preparing to look at the biological response of these patients. The second study involves mood, noradrenergic, and neuroendocrine changes in manic and depressed subjects in response to intravenous clonidine infusions. The third study looks at changes in mood, memory, sleep patterns, and electrophysiology in depressed patients in response to ACTH and its centrally acting analog, ACTH 4-9.

PHS-6040
(Rev. 10-76)

Project Description

This project covers three pharmacologic studies in depression using different classes of drugs, which interact with different neurotransmitter systems. Amphetamine, a drug with well-known activating and mood-altering properties, stimulates release of dopamine and norepinephrine. Clonidine is an antihypertensive agent with central noradrenergic agonist actions. ACTH is a pituitary hormone with actions on both the adrenal cortex and receptors in the brain itself. Organon 2766 is an analog of ACTH 4-9, a fragment of ACTH known to act on CNS receptors, but not on the adrenal.

In the initial phase of the amphetamine study, 18 patients with unipolar and bipolar depression were given 20 mg intravenous amphetamine. The group response consisted generally of activation and mood elevation, although there was considerable heterogeneity in direction of mood change and degree of emotionality following drug administration. Bipolar depression was significantly related to mood elevation following drug. A report based on this phase of the investigation has been submitted for publication. At the present time, we are analyzing blood samples drawn during the procedure to investigate correlations of biological response and mental status change. Changes in MHPG levels before and after amphetamine have been reported to correlate with both amphetamine and tricyclic antidepressant response; cortisol and growth hormone levels have also been reported to vary with certain aspects of amphetamine response. The present phase of the study will attempt to corroborate these findings as well as to relate the reactive subtypes found in the clinical study to biological parameters.

Clonidine is a drug with known noradrenergic agonist actions. A significant portion of these actions is presynaptic. It has been demonstrated in animals, for example, that systemic clonidine infusions decrease firing of the locus coeruleus, a noradrenergic center in the brainstem. This center is involved in the mediation of activation and anxiety levels, and success in treating opiate withdrawal with clonidine is presumably due to the drug's moderating action on its activity. Since there are indications of increased noradrenergic activity in mania and anxious depressions, we felt that the action of clonidine in these conditions would be of interest. The protocol involves intravenous administration of 2 micrograms/kg to depressed and manic patients. Mood and anxiety levels are monitored during the procedure; blood samples are drawn for measurement of noradrenergic metabolism as reflected in MHPG. In addition, in collaboration with Dr. Larry Siever, levels of growth hormone, luteinizing hormone, and prolactin are being measured at intervals throughout the procedure. Initial data indicate a possible anti-manic effect, as well as changes in anxiety level in depressed subjects. Preliminary blood analysis suggests a possible blunting of growth hormone response to the drug, as compared to normal controls.

The ACTH study is the final phase of an investigation initiated by Dr. Victor Reus. The study was set up to investigate the effects in depressed patients of ACTH and an analog of the ACTH 4-9 fragment,

a compound known to be active at sites in the brain (particularly hippocampus), but devoid of adrenocorticotrophic effects. Dependent measures included mood ratings, a verbal and visual learning battery, evoked potential studies, sleep studies, and blood assays of cortisol and norepinephrine. Data collection for the study has now been completed, and most of the results have been analyzed. Patients showed no drug effect on mood or memory performance. ACTH administration was found to disrupt REM sleep, while ACTH 4-9 disrupted slow wave sleep. ACTH and ACTH 4-9 were found to have effects on the attentional response of subjects to lights and tones, as measured by average evoked response of the EEG. While ACTH enhances the attentional component of the AER compared to placebo, ACTH 4-9 diminishes it to the point of eliminating the usual attention-stimulus intensity gradient. It is notable that the most prominent effects of these drugs appeared at the longest times after administration, raising the question for future studies of whether peak effects on mood and memory, if any, would appear in a later time frame than the one currently used. In connection with Dr. Dave Pickar, we are now investigating possible interactions between ACTH and the endogenous opiate systems.

Publications:

Silberman, E.K., Reus, V.I., Jimerson, D.C., Lynott, A.M., and Post, R.M.: Heterogeneity of amphetamine response in depressed patients. Am. J. Psychiatry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00502-02 LPP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Atypicality in Major Depressive Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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1.5	1.0	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To explore the possibility of an important atypicality dimension within the group of primary, endogenous depression, we have constructed a rating scale for atypical depressive illness. Forty-four NIMH patients were rated, all meeting Research Diagnostic Criteria for primary, major depressive illness. Atypicality in this group was characterized by lack of encapsulated episodes, interpersonal difficulties, evidence of narcissistic character disorder, and high anxiety and somatization. Though atypical patients were younger than typical, they were hospitalized significantly more often. Biologically, they had smaller variance in two measures of norepinephrine metabolism, as well as lower levels of platelet MAO. Typicals were significantly more likely to have an antidepressant response to sleep deprivation than atypicals. Thus, atypicality as a dimension within primary, major depression may have important theoretical and clinical implications.

PHS-6040
(Rev. 10-76)

Project Description

The initiation of this project was based on our observation of considerable heterogeneity of clinical presentation within the group of patients meeting Research Diagnostic Criteria for primary, endogenous, major depressive disorder. We reasoned that this clinical diversity may contribute significantly to the biological and prognostic heterogeneity within this group. To investigate the possibility of important sub-types or of an important atypicality dimension within this group, we have constructed a rating scale for atypical depressive illness. The scale is designed to measure degree and type of divergence from a classical syndrome of discrete episodes of autonomous mood and vegetative symptoms.

Forty-four NIMH patients were rated, all meeting Research Diagnostic Criteria for primary, major depressive disorder. In addition, 91% of these met criteria for endogenous depression as well. There were 21 men and 23 women; 28 patients were bipolar and 16 unipolar. In a preliminary session, global atypicality ratings on a zero to ten scale were derived by consensus of trained raters from the psychiatry, social work, and nursing services. Subsequently, the same group consensually rated patients on each of 24 items, to give a total scale atypicality score. Scale items assessed degree of autonomy of symptoms, vegetative signs, personality disorder, neurotic symptoms, thinking and perceptual disturbances, past course of illness, and premorbid adjustment.

Atypicality ratings derived by the two different methods were highly correlated ($r = .83$, $p < .0001$). Atypicality was characterized by relative lack of encapsulated episodes, disturbed interpersonal relationships, narcissistic entitlement, manipulativeness, and prominent anxiety and somatization. Atypicality scores were not related to severity of depression. Atypical patients were significantly younger than typical patients ($p < .002$). Bipolar I patients were significantly more typical than either bipolar II or unipolar patients ($p < .05$); however, the difference no longer reached significance when age was covaried out. Atypical patients were hospitalized significantly more often ($p < .02$) than typical patients, even though the typicals tended to be faster cyclers than the atypicals. Biologically, the typical group had significantly less variance in measures of MHPG metabolism as reflected in urinary MHPG ($p < .05$) and spinal fluid norepinephrine ($p < .03$). While the atypicals tended to have higher MHPG levels, they had lower platelet MAO ($p < .056$). Typicals were significantly more likely than atypicals to have an antidepressant response to sleep deprivation than atypicals ($p = .048$).

The preliminary data cited above suggest that atypicality may be an important factor within primary, endogenous depression. It is not yet clear whether atypicality is best conceptualized as a subgroup or as a dimension within depression. Because in a research setting we are dealing with a selected population, the interpretation of lower age and more frequent hospitalizations in the younger group is not yet clear. On the other hand, it may be that depression presents in a more varied and atypical manner

when the patient is young, but evolves into the classic syndrome later in the patient's course. On the other hand, it may be that the atypical group is distinct from the typicals, with a greater propensity for regressive episodes and hospitalization. It is notable that within our group, atypicality was represented by features of narcissistic character disorder and disturbed interpersonal relationships, rather than by schizoid trends or thinking disorders. Again, this may be a result of screening that would not apply to the general depressive population. There was an indication that the bipolar I group was highly typical, while the bipolar II and unipolar patients were equal in atypicality. Because age and polarity were confounded in our patient population, it was impossible to reliably test for an association between typicality and polarity.

Biologically, the trend toward higher MHPG and lower variance in norepinephrine metabolism can be placed in context of previous biological studies. Generally speaking, lower MHPG has been associated with more classical and more severe forms of depression. While low platelet MAO has also been associated with more classical (bipolar) depression, it is also associated with a group of people within the general population who have high levels of personality disorder, particularly sensation-seeking and sociopathy. Thus, within our group, atypical patients tended to have low MAO, while typical patients had either high or low MAO.

While the above data were gathered retrospectively by consensus, plans are now under way to rate subjects as they enter the hospital. Working with a fresh, common data base will allow higher reliability than retrospective consensus ratings. Information gathered in this way will enable us to examine correlates of varying types of atypicality, rather than just total scale scores. In addition, we are collecting data on course of illness and response to treatment after NIH hospitalization.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00503-01 LPP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Human clinical studies of attention disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Fritz Dreifuss	Professor of Neurology	University of Virginia	

COOPERATING UNITS (if any)
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3.0	1.75	1.25

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This research comprises three related areas of investigation concerned with specifying neuropsychological factors underlying clinical conditions in humans in which disturbed attention is a major symptom. A major emphasis is on (1) illuminating the nature of brain stem pathophysiology, if any, in such entities as petit mal or absence epilepsy, infantile autism, schizophrenia and related diseases; (2) an additional major emphasis is on extending the neurobehavioral analysis of attention loss in absence epilepsy so as to facilitate developing alternative treatment strategies for such patiients. Both of these projects form part of a larger effort which is aimed at (3) developing a comprehensive and systematic taxonomy of attentional disorders in humans. This latter study will eventually comprise study of patients with cerebral lesions, seizures, dementing diseases and metabolic illnesses of the brain.

PHS-6040
(Rev. 10-76)

Project Description:

1. Brain stem mechanisms in attention impairment.

Current approaches to the neuropsychology of attention impairment have emphasized that the system responsible for the maintenance of attention (consciousness within the brain is most likely represented as a variety of levels of the neuraxis). From an evolutionary point of view, it is clear that the capacity for sustained attentive behavior is present in many species which do not possess more than a rudimentary forebrain or telencephalon. MacLean's analysis of the R-complex within the human brain leads to the view that this "clump of ganglia," which constitutes virtually all of the reptilian brain, can support a variety of ritualistic, repetitive behaviors which could be characterized as sustained and attentive. Evolution progressed and the brain developed additional complexity and volume. Additional capacity for attentive behavior was thus overlaid on the more primitive, although in many aspects thoroughly adequate, brain stem system of the reptile. Therefore, although the system for maintenance of attentive behavior in the human (or higher primate) includes limbic and neocortical components, the brain stem remains a key component and possibly the keystone of the entire system. Authors such as Hughlings Jackson and Penfield and Jasper recognized this in their conceptions, respectively, of "highest level seizures" and the "centrencephalon." In their theorizing, consciousness was either localized in or regulated by deep brain stem structures. Without reviewing all of the evidence that led to those views of the hierarchical organization of attention and consciousness within the brain, we nevertheless point to the extremely deleterious effects on such capacities of small lesions in the brain stem region of the third and fourth ventricles. In the last 10 years a new technological refinement of evoked potential methodology has made possible an other-than-theoretical exploration of the role of brain stem structures in certain clinical states. This "far field" technique makes it possible to assess the integrity of auditory (and somatosensory) relay nuclei within the brain stem of humans. Although the technique has probably had most utilization in the diagnosis of demyelinating disease, it has also been used in the study of other neurological and, recently, psychiatric disorders. There may or may not be any specific interest in these sensory systems (auditory, somatosensory) in studying a particular clinical entity (i.e., absence seizures, infantile autism); nevertheless, the possibility of evaluating the functional integrity of certain systems within the brain stem is extraordinarily valuable and many clinical investigators are using these techniques. We have published work indicating that there are disturbances (prolonged transmission time) in the processing of auditory information in the brain stem in infantile autism. We have also shown that in absence seizures (spike-wave activity), both naturally-occurring and experimentally-induced, there may be perturbations of auditory brain stem functioning. We are planning to continue such studies with these patient groups and others once our facilities have been developed in the ACRF.

2. Neurobehavioral studies in absence epilepsy.

We have for a number of years been studying the absence attack in patients with petit mal/centrencephalic/absence seizures (the terms are more or less interchangeable) as model state to understand the phenomenon of consciousness/attention. Some of these studies have involved comparing the behavioral capacities of patients suffering from petit mal--as opposed to focal seizure disorders; other studies have involved detailed comparison and contrast between the behavioral and the electroencephalographic symptoms/signs of the disorder. Most recently these investigations have: (1) used evoked potentials in the visual and auditory modalities as indices of the sensory effects of generalized seizure activity of the symmetrical and synchronous spike and wave (sw) variety, and (2) examined changes in the EEG power spectrum prior to sw bursts as prodromal signs which may be used to predict (and ultimately to control) sw bursts. We propose to continue this line of neurobehavioral investigation, using event related potentials of various types as well as other behavioral and physiological tools, to refine further our understanding of the nature of altered consciousness in absence (petit mal) epilepsy.

3. A taxonomy of attentional disorders.

The goal of this project is to develop a comprehensive and coherent account of the relation between symptoms of altered or disturbed attention or consciousness as they appear in various clinical entities, the other behavioral and clinical characteristics of the several disorders, and the specific central nervous system damage or disturbance in each disorder. The attentive capacities of the patients will be assessed by a number of measures comprised within the GAT (generalized attention test) which is an outgrowth of the CPT (continuous performance test) a measure of sustained visual attentive behavior. The ultimate goal will describe the precise attentive deficit (as opposed to cognitive losses) and the nature of the neuropathophysiology associated with each of the following clinical entities:

cerebral cortical lesions (frontal, parietal or temporal lobe)
centrencephalic/absence epilepsy
schizophrenia
infantile autism
dementing diseases (Alzheimer's, Korsakoff's, Huntington's).

We will attempt, as well, to relate these changes where possible to standardized measures of mnemonic and other cognitive function, and to autonomic indices of attention, arousal, and habituation.

We have run a small group of schizophrenic patients through our laboratory procedures (i.e., CPT, brain stem auditory evoked potentials, various tests of cognition and memory, autonomic indices of attention, etc.) During the course of the next year we hope to recruit additional cases from other diagnostic categories into this taxonomic study.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00504-01

PERIOD COVERED
October 1, 1980 to September 1, 1981

TITLE OF PROJECT (80 characters or less)
Experimental models in the rhesus monkey of generalized seizures of the absence type.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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0.6	0.6	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Generalized seizure activity with the electrographic appearance of absence epilepsy (bilaterally symmetrical and synchronous paroxysmal three-per-second spike and wave discharges) can be elicited in the monkey by a variety of methods. These include electrical stimulation of various locations within the brain, injection of convulsant drugs and other substances, and administration of compounds which may alter normal inhibitory mechanisms within the cell. Model seizure states created in these ways are studied in order to test hypotheses about pathophysiological seizure mechanisms, sensory processing and attentional capacities during absence seizures, effects of spike-wave activity on cellular activity, and effects of techniques or maneuvers which may modify or reduce convulsive activity. Most recently this project has involved the following work: we studied the (paradoxical) seizure inducing effects of a GABA-enhancer and the effects on auditory brain stem evoked potentials of generalized seizures induced by injection of pentylenetetrazol.

PHS-6040
(Rev. 10-76)

Project Description

γ-vinyl GABA and γ-acetylenic GABA are two recently synthesized compounds whose metabolic effects include the blocking of the enzyme action responsible for the metabolism of the inhibitory neuro-transmitter GABA. The accumulation of GABA thus produced should have an anticonvulsant action, and so it does, at moderate doses of these compounds. However, as the dose is increased, there is a paradoxical rebound effect and animals treated with large quantities of either γ-vinyl or γ-acetylenic GABA have shown paroxysmal seizure activity. And of interest to us is the fact that the seizure activity is not the clinically obvious generalized tonic-clonic variety. Instead, although widespread spikes and spike-wave patterns may be seen, there may be few clinical signs. Such an effect is reminiscent of absence seizures (staring spells) in human centrencephalic epilepsy. We are in the process of exploring the utility of these compounds for producing model seizures of the petit mal variety in the monkey.

We have also induced generalized seizure activity in rhesus monkeys, reflected in both clinical and EEG manifestations, by systemic administration of pentylenetetrazol. Brain stem auditory evoked potentials (BAEP) were recorded from indwelling epidural electrodes at the vertex of the skull as well as from electrodes implanted along the primary auditory pathway in the brain stem (inferior colliculus). Several components of the complex "far field" vertex potential showed increased latency and decreased amplitude during ictal episodes as compared to control periods both pre-drug and post-seizure. Similar changes were seen in direct recordings from brain stem auditory structures. The parallel recording of "far field" (vertex) potentials and "near field" (brain stem auditory pathway) potentials appears to be a fruitful approach. The direct recording from brain stem auditory structures adds reliability and temporal resolution to the findings. Thus, in contrast to the vertex potential which requires several hundreds, or even thousands of stimulus repetitions, only a few samples are necessary to obtain reliable waveforms from direct brain stem recordings. Consequently, the grain and the resolution of the experiment can be enhanced, and various periods of pre-, during- and post-ictal stages as well as phases of gradual recovery can be assessed. The analysis of small consecutive samples revealed profound BAEP changes not only during the ictal period but immediately following the seizure activity. There was also marked fluctuation of suppression and potentiation during the post-ictal recovery period.

The significance of this experiment lies in the fact that it provides direct evidence of brain stem involvement in generalized seizures and contributes to the current efforts to produce an accurate primate-based model of the pathophysiological processes in abence epilepsy. We will be continuing with this experimental program.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00505-01 LPP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Brain lesion and state change effects on visual attention

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard K. Nakamura	Senior Staff Fellow	LPP	NIMH
OTHER:	Allan F. Mirsky	Chief	LPP	NIMH
	Richard Coppola	Engineer Officer	LPP	NIMH
	Eva Bakay Pragay	Research Psychologist	LPP	NIMH
	Mortimer Mishkin	Acting Chief	LN	NIMH
	Robert Desimone	Staff Fellow	LN	NIMH
	H. Enger Rosvold	Research Physiologist	LN	NIMH
	David Friedman	Staff Fellow	LN	NIMH
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	Masanori Ito	Fogarty Scholar	LCM	NIMH
	Sumio Suda	Fogarty Scholar	LCM	NIMH
	J. Christian Gillin	Research Psychiatrist	BPB	NIMH
	Richard Wyatt	Director	SMRC	NIMH
	William Freed	Staff Fellow	SMRC	NIMH
	John Morihisa	Clinical Associate	SMRC	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project consists of four related areas of investigation, all concerned with the analysis of mechanisms of attention. Special emphasis is placed on the use of lesion and/or biochemical techniques to elucidate mechanisms involved in visual attention. The four areas are: 1. attention and cerebral mechanisms of visual behavior; 2. pharmacological mechanisms of attention; 3. brain activity in inattention: local cerebral glucose metabolism in sleep; and 4. the design of a general attention task.

Project Description:

1. ATTENTION MECHANISMS AND VISUAL BEHAVIOR

We have found that large nonvisual lesions of cerebral cortex in the monkey cause permanent blindness. The basic preparation is as follows: one hemisphere is visually differentiated by combined optic tract transection and forebrain commissurotomy. The other hemisphere has all cortical areas removed except for the striate, prestriate and inferior temporal visual areas. Animals prepared in this way were functionally blind for over a year despite anatomical evidence of an intact visual system. We have been analyzing this phenomenon in an effort to determine the role of nonvisual cortex in visual behavior and to establish the nature of higher processing in the visual system. The major results indicate that we are beginning to elucidate the role of attention in sensory processing.

We have examined the single neuron responses to visual stimuli in the visual systems of our blind monkeys. Results of over 400 neurons studied to date in the blind animals, compared to over 200 neurons in normal animals, indicate that visual responses of the blind are near normal in both striate and prestriate areas. Preliminary data from inferior temporal cortex, on the other hand, suggest that the blind animals show less specificity of neuronal response than seen in the normals. These data imply that in the absence of feedback from higher cortical structures and in the absence of behavioral feedback, the visual system continues near normal processing through to the prestriate cortex. Only at the level of inferior temporal cortex does the effect of such losses of feedback change cellular responses to visual stimulation. This in turn suggests that the effects of attention on neuronal responses will not be significant in areas earlier in the visual pathway than inferior temporal cortex.

In an effort to determine the critical cortical zones of this blindness phenomenon, the large nonvisual ablation has been subdivided into three parts: the sensorimotor cortex, the limbic cortex, and the polysensory cortex. These areas have been ablated in separate groups of monkeys and the only lesion which produces a blindness effect is the polysensory cortical lesion. This area consists of dorsal prefrontal cortex, inferior parietal cortex, and superior temporal cortex (including insula). It is of considerable significance to us that a neglect or inattention syndrome follows the ablation of any portion of the polysensory area, for this suggests that our animals cannot see because they cannot attend to the visual modality.

The absence of effect of the sensorimotor lesion creates confidence that the blindness is not simply the result of a disconnection of visual input from motor output. In the original chronic blindness preparation, the nonvisual lesion is placed in one hemisphere, the optic tract to the other is cut and the forebrain commissures are divided. If, however, the forebrain commissures are left intact, then there will be a period of blindness lasting approximately 10 to 40 days. This period is followed by a recovery

of visual function which permits not only visual guidance to food, but discrimination of visual patterns as well.

This recovery of visual function, when a path of communication is left between the hemispheres indicates that the blindness is the result of a disconnection of vision from higher cortical processing areas. Combined with earlier results, we can be more specific and say that the blindness is caused by a disconnection of visual areas from polysensory (attentional) areas of the brain. Thus a connection between visual and attentional areas appears to be critical before visual behavior can proceed in monkeys.

2. PHARMACOLOGIC MECHANISMS OF ATTENTION:

Striatal dopamine has been implicated as an important transmitter in the attention-arousal system. Areas A9 and A10 (substantia nigra pars compacta and the ventral tegmental area) have been shown to be major sources of dopamine for the brain in general and the cerebral cortex in particular. In rats, lesions of areas A9 and A10 have been associated with inattention or neglect, tremor, transient aphasia and adypsia, and a deficit on the delayed alternation task. Little is known about the effects of such ablations in the monkey though in man cell loss and reduced dopamine in these areas have been associated with Parkinsons disease.

We have therefore begun a study of A9 and A10 lesions in monkeys. We are looking for deficits in attention, motor performance, delayed alternation, food and water intake, and reduced dopamine levels in caudate and frontal cortex. Results thus far indicate that lesioned monkeys show attention losses in the somatosensory and auditory modalities but not in vision. They also show little change in motor performance, food and water intake, and behavioral performance of delayed alternation or visual discrimination.

Because of our interest in striatal dopamine, we have begun a study with the division of Special Mental Health Research to investigate the possibility of transplanting dopamine secreting tissue into the brains of monkeys that have been previously deprived of striatal dopamine with unilateral 6-hydroxydopamine ablations. Objectives of the research are to: a) look at the effects of unilateral ablations of dopamine systems b) examine the practicability of brain tissue implants in the monkey, and c) see if behavioral changes following dopamine ablations can be reversed with transplanted tissue. Preliminary data indicate that it is possible to transplant fetal substantia nigra tissue into a monkey brain and get both growth and dopamine secretions.

3. BRAIN ACTIVITY IN A STATE OF INATTENTION

Major clues to the systems involved in attention might be derived from the study of natural states of reduced attention such as sleep. In collaboration with the Laboratory of Cerebral Metabolism, the Laboratory of Neuropsychology, and the Sleep Laboratory, we have been studying cerebral metabolism of monkeys in slow wave sleep and wakefulness.

Over the past year, a total of eight monkeys have been examined, four experimental animals in slow wave sleep and four control animals that were kept awake. The major finding has been that the animals in sleep show an overall reduction in cerebral metabolism of about 30%. Further, we have been unable to find any brain structure which shows, on average, higher activity in slow wave sleep that in the awake state. Hypnogenic center theories, which postualte a brain area which actively keeps an animal in sleep, are therefore not supported. The loss in attention which accompanies slow wave sleep thus appears to be the result of a general diminution of brain activity.

We will examine next the effect of paradoxical or REM sleep on brain activity. REM sleep is of particular interest because the organism is in a state of total inattention while at the same time the brain is electrographically very similar to the awake brain. Thus, the brain structures controlling the difference in attention stages may be more readily revealed.

4. DESIGN OF A GENERAL ATTENTION TASK:

While the foregoing studies may reveal important clues to the nature of attention, they all represent inherently indirect approaches because in none is a systematic attempt made to isolate the attentional process while controlling for other processes such as sensory input and motor output. If sensory input and motor output were held constant while attention was made to switch from one aspect of the sensory input to another, then it would be possible to study directly the mechanisms of attention.

We have begun the process of designing a general purpose test which will allow such a direct examination of attention. Included are additional design features such as: a) the parameters of the task will be usable for both humans and monkeys; b) it will accommodate both electrophysiological and behavioral analysis by permitting quantification of attention changes and allowing precise timing of brain events associated with these changes; and c) information will be transmitted through the visual system to take advantage of the considerable information available on visual processing.

Implementation of this task has begun and feasibility studies will be performed this year on both monkeys and humans.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00506-01 LPP

PERIOD COVERED
October 1, 1980 to September 1, 1981

TITLE OF PROJECT (80 characters or less)
Attention-related neurons in the brain of the rhesus monkey.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Eva Bakay Pragay	Research Psychologist	LPP NIMH
OTHER:	Allan F. Mirsky	Chief	LPP NIMH
	Ralph U. Esposito	IPA detail	Boston University

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	2.2	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with an analysis of the activity of nerve cells in that system within the primate brain which is necessary and responsible for the process we refer to as attention. Monkeys trained to perform visually-guided go-no go discrimination tasks are tested whilst extra cellular recordings are made from brain regions thought to be part of an attentional system. The most recent study in this series examined structures in the forebrain.

We have learned that the dorsolateral prefrontal cortex as well as the area around the cingulate sulcus and the rostral neostriatum is well supplied with cells which respond to the manipulation of attention. This was accomplished by altering the reward value of the conditioned stimulus or by varying the waiting period preceding stimulus onset. These attention-related units have functional properties similar to those found in the brainstem reticular formation in previous studies by Bakay Pragay, Mirsky, Ray and colleagues. However, the attention cells appear to be more numerous in this region of the forebrain than in the brain stem. Moreover, the degree of plasticity we observed was apparently higher in the forebrain than in the brainstem reticular formation.

Project Description:

In an effort to describe at a cellular level the brain system in the primate necessary for attentional behavior, we have developed a monkey preparation that permits such study. Extra cellular recordings are made from trained animals while they perform on discrimination tasks requiring visual attention. In earlier work we described "attention-related" cells in the mesopontine region of the brainstem. Later work has been aimed at forebrain structures including the dorsolateral prefrontal cortex (around the sulcus principalis), the upper bank of the rostral portion of the cingulate sulcus and the rostral neostriatum. This description focuses on the results of that study.

As in prior studies, the task required the animal to press a "hold" button for 2 seconds in order to turn on a "cue" button; the letter was transilluminated by either a red ("go") or a green ("no go") cue-light. In the go trials, the animal had to release the hold button and press the cue button within 1 second. In the no go trials, it had to maintain pressure on the hold button for another second. In the basic task, both correct go- and no go trials were rewarded.

Only task-related units were studied systematically. The majority of units (50 out of 57) showed changes with both go and no go trials. This so-called Type II activity is contrasted with Type I activity which occurs on go trials only. In the basic task, Type II activity could be classified into the following sub-groups: (1) post-stimulus, pre-reinforcement change; whether a relatively brief phasic or more tonic change, the activity declined before the onset of reinforcement (N=16). (2) anticipatory activity, occurring in the intertrial interval: this was usually in the form of a gradual increase in firing rate of the cell preceding, and occasionally following, the stimulus onset (N = 20). (3) a complex pattern of activity, consisting of a post-stimulus and a peri-reinforcement change, with a clear-cut separation between the two bursts of activity (N = 10). (4) peri-reinforcement activity only (N = 4). The attention-related property of the Type II units was tested by varying the within-task conditions. These included: a) reward for both correct go and no go trials (basic task); b) non-reinforcement for correct no go trials (NRNG); c) varying the length of the fixed intertrial interval (ITI): 1 sec, 2 sec (basic task) and 3 sec (or 4 sec). In addition, 2 extra-task-conditions were applied: visual stimuli (including the task-stimuli) without access to task or reward, and non-contingent delivery of reward. The NRNG condition and the extra-task administration of stimuli represented manipulation of attention through variation of the reward value of the stimuli. Variation of the length of the ITI manipulated attention by varying the possibility of development of a preparatory set.

Changing the reward-value of the stimulus and the length of the ITI affected performance. This was reflected in changes in reaction time (RT) of the instrumental motor response on the go trials as well as in late responses, and omission errors. Increasing the length of the ITI to 3 or 4 seconds

decreased RT; decreasing the ITI to 1 sec increased RT both of the "lift" response and of the "hit" response, and occasionally to omission errors or temporary inhibition of performance. The NRNG condition decreased RT. Among these conditions, the decrease of ITI to 1 sec brought about the most marked RT changes and decline of performance. General motor restlessness was often observed with the NRNG condition and with increases in the ITI.

With respect to the effects of these manipulations of attention on unit activity, a number of observations were made. In the NRNG condition, all peri-reinforcement spike discharges on no go trials terminated. In the peri-stimulus-activity, various degrees of loss of the original reinforced response or the reverse form of response were seen (i.e., from increase to decrease). In all, 33 units (out of 40 units so tested) were changed by NRNG. The vast majority of units also responded to the non-contingent delivery of reward. Varying the ITI affected 26 of the 32 units tested. Increasing the ITI prevented such activity and in a number of cases disrupted task-related unit behavior. When manipulation of attention changed the RT, there was a parallel change in the latency of unit firing. Extra-task administration of the CS rarely evoked any systematic change.

No substantial regional differences in cell type or behavior were seen in the various areas of the forebrain we examined. However, the cingulate sulcus may be especially rich in anticipatory units as well as in units responding to the non-contingent delivery of reinforcement. In contrast, such units were less often seen around the sulcus principalis.

This study is an important step forward in our program of attention research. It represents a second step in the line of exploring various brain regions, by means of extra-cellular unit recording techniques in a go-no go task. It also represents an expansion of our experimental techniques for manipulating and describing the attention-functions of Type II units.

In the coming year we are planning as well to extend our investigation of brainstem attention-reward related cellular activity by examining the role of monoaminergic systems that constitute neurochemically specific components of the brainstem reticular formation. We are particularly interested in the nigrostriatal-mesotelencephalic dopaminergic systems and the dorsal noradrenergic projections of the pontine locus coeruleus. A wealth of experimental data, derived from both animal and human studies, suggests that these catecholaminergic systems may comprise an integral part of a central system that enables organisms to selectively attend and respond to significant and potentially rewarding stimuli. Furthermore, a great deal is known concerning the anatomical, electrophysiological, and neuropharmacological characteristics of these systems, perhaps more than any other central neuronal system. This will provide a firm foundation for a functional analysis of these systems at the cellular level.

Our initial aim will be to search for task-related cells at the sites of origin of these systems, specifically areas A9, A10, and A6 according to the nomenclature of Dahlstrom and Fuxe.

It is noteworthy that recent immuno-histochemical studies have demonstrated that the anterior cingulate and rostral neostriatal regions receive prominent, and heretofore unsuspected projections from areas A9 and A10, respectively. The functional characteristics of these cells will be compared and contrasted, according to our usual analysis, with the task related activity of the forebrain cells.

Then by systemic, and perhaps intraventricular, administration of various catecholamine agonists and antagonists we will examine the neuropharmacological properties of these cells in relation to specific attention-reward related aspects of cell activity. Finally, microiontophoretic application of relevant agonist/antagonist agents should allow for a fine grain analysis of pre-synaptic, post-synaptic and autoregulatory cellular events, as they relate to various aspects of task activity. These studies should provide a basis for our eventual study of the functional integration of brainstem and forebrain mechanisms in the regulation of attention and reward related behavior.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00671-12 LSES

PERIOD COVERED

October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Social Origins of Stress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leonard I. Pearlin	Research Sociologist	LSES NIMH
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COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The research examines the relationship between social experience and psychological well-being. It looks at the enduring problems people encounter in their daily lives as workers and family members, at the gain and loss of roles as the life-cycle unfolds, and at sudden, unexpected crises that often confront people. Each of these types of experience is examined in relation to symptoms of emotional distress. Data for the study were collected at two times. In 1972 a sample of 2300 people, representative of the Chicago Urbanized Area were interviewed, and a sub-sample (1200) of this group was reinterviewed in 1976-77. Analyses have been made to identify the events that are particularly likely to result in emotional distress, and to understand how people use psychological and social resources to cope with the problems and vicissitudes of life.

PHS-6040
(Rev. 10-76)

Project Description:

This is a longitudinal study of social stress with two interviews conducted at an interval of about four years. The first wave of interviews was carried out in 1972 with a representative cluster sample of 2300 adults in the urbanized area of Chicago. These interviews sought information about three features of people's lives: 1)the chronic, often low-keyed, everyday problems that people encounter in their occupational, marital and parental roles; 2)the coping strategies people employ in dealing with life-problems; and 3)symptoms of emotional stress, anxiety and depression. Then in 1976-77 a second wave of interviews was conducted among a sub-sample of 1200 people drawn from those originally interviewed. Many of the questions that initially asked about chronic role problems, coping, and psychological distress were repeated, thus providing longitudinal information about stability and change on these dimensions of people's lives. Newly constructed questions sought information about key life-events that occurred since 1972. One type of event is represented in the many changes and role transitions that are normative aspects of the life-cycle. A second type of event is represented in the more unexpected, non-normative disruptions of important roles and activities, such as divorce, serious illness, involuntary unemployment, and other life cirses. It is the long-range goal of the research to reconstruct from this information those elements of social experience that importantly affect mental health and to identify those coping behaviors and social supports that serve as barriers to psychological harm.

During the past year we engaged in two major analytic tasks. One was conducted in conjunction with the Institute of Medicine of the National Academy of Sciences. The Institute endeavored to bring together experts who could assemble the significant knowledge concerning the relationship between stress and health. The principal investigator was co-chair of the panel on life events and health. In this capacity an intensive review was made of life-events research, including that which is part of the principal investigator's own study. The review revealed several substantive and methodological problems in investigations of life events and health.

One problem is that life events are often studied apart from their social contexts in which they arise; that is they are treated as though their occurrence in the lives of people is a random phenomenon. An epidemiology of events is needed in order to understand how eventful experience may be anchored to the location of people in the society. A second problem, one that is methodological, concerns the confounding of events with presumed effects. The standard scales measuring eventful experiences typically include items involving changes in the well-being of people, such as injury or illness. These scales are then related to outcome measures which themselves often include elements of physical or mental health. Under these conditions of contamination, it is difficult to assess what really has been learned of the relationship between life events and health. Finally, there is confusion regarding the best fit between the

goals of the research and the choice of research strategies for studying life events. Several options are open to the researcher: broad scale measures of total life events; clusters of events, such as undesireable; single events, such as death of a spouse; events that affect large collectivities, such as community disasters; or, finally, one may choose to examine special at-risk groups, such as coal miners. At this time there is little understanding of the unique yield to life-events research of each strategy.

A second major task during the past year involved the refinement and extension of previously established lines of inquiry. Specifically, work was continued on an empirically based model of the stress process. The model observes how life events, chronic life strains, coping and social supports converge in the creation of stress. It was earlier observed that life events can intensify the more persistent life strains and that such intensification, in turn, erodes self-esteem and mastery. When these self-concepts are diminished people become more vulnerable to stress. Coping and social supports can intervene in this etiologic process in an efficacious manner. However, they do not directly intervene on stress itself, but on its antecedents. That is, these moderators buffer the stress by vitiating against the elevation of life strains and by supporting and sustaining positive self-concepts. It is in these indirect ways that coping and support minimize stress. The functions of social supports are particularly specialized in these regards, for their effectiveness is exclusively limited to buttressing self.

The principal investigator conducted his work in the past year as a visiting professor in the Adult Development and Aging Program at the University of California in San Francisco. The purpose of the year was to become familiar with the theories of development in aging. In the coming year, it is anticipated that life course and aging perspectives will be brought into the analyses of the data. These perspectives will be especially useful if there is a third interview. Preliminary planning of a longitudinal extension of the study will be begun in the next year.

Publications:

Pearlin, Leonard I.: The social epidemiology of drinking behavior: Some implications for the work place. National Institute of Alcohol and Alcoholism Research Monograph Series, Washington, D. C., Government Printing Office, 1980.

Menaghan, Elizabeth, Pearlin, Leonard I., Lieberman, Morton A. and Mullan, Joseph T.: Life events, world conditions and adaptations. In: Sarnoff A. Mednick and Michele Harway (Eds.): Longitudinal Research in the United States. Boston, Nijhof Press (In press).

Dohrenwend, B. P. and Pearlin, Leonard I.: Report of the Panel on Life Events from the Committee for Research on Stress in Health and Disease. Institute of Medicine, National Academy of Sciences, Washington, D. C. Pp.150-169, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00672-16 LSES

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Social Psychological Correlates of Occupational Position

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	C. Schooler	Research Psychologist	LSES	NIMH
	J. Miller	Research Sociologist	LSES	NIMH
	K. Miller	Research Sociologist	LSES	NIMH
	A. Naoi	Visiting Scientist	LSES	NIMH
	K. Slomczynski	Visiting Scientist	LSES	NIMH
	R. Schoenberg	Research Sociologist	LSES	NIMH
	C. Schoenbach	Social Science Analyst	LSES	NIMH

COOPERATING UNITS (if any)

None

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Laboratory of Socio-environmental Studies

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12.5	6.0	6.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this study is to assess the reciprocal effects of occupational conditions and psychological functioning (in particular, values, self-conceptions, social orientation, and intellectual flexibility). Structured interviews were conducted in 1964 with a sample of 3101 men, representative of all men employed in civilian occupations throughout the United States. The study was extended into a longitudinal study in 1974, with the re-interviewing of a randomly-selected one-fourth of the original sample, together with their wives and, where appropriate, one of their children. Replications of this research have been carried out in Poland and Japan.

PHS-6040
(Rev. 10-76)

Project Description:

The principal goal of this research is to assess the relationships between people's job conditions and their psychological functioning. The research began in 1964 with structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations throughout the United States. These interviews were conducted to Melvin Kohn and Carmi Schooler's specifications by the National Opinion Research Center (NORC) of the University of Chicago. In 1974, NORC conducted follow-up interviews, again to Kohn and Schooler's specifications, with a randomly selected one-fourth of the men who had participated in the original survey. Wherever a man was found to be presently married, a nearly identical interview was separately conducted with his wife. And wherever a man had one or more children in the age-range 13 through 25, a similar interview was conducted with a previously selected child.

One major purpose of the follow-up study has been to provide more definitive data about causal processes than could be provided by a single cross-sectional survey. With these data, the investigators have attempted to assess the magnitudes of the reciprocal effects of job conditions and various facets of psychological functioning. The study of wives was designed to ascertain whether job conditions affect men and women similarly. (On this, see the 1977-78 report.) These data should also enable the investigators to assess the effects of men's job experiences on their wives' psychological functioning and of women's job experiences on their husbands' psychological functioning, in each case taking account of the individual's own job experiences. The study of the children was designed for exploratory analyses of the effects of parental experiences, values, and practices on their children's psychological development, as well as of the children's own educational and occupational experiences on their own psychological development.

During this past year, the major research efforts have been addressed to seven objectives: (1) Using the longitudinal data for men, analyzing the reciprocal effects of job conditions and psychological functioning. (2) Using the data from both the men and their wives, analyzing the reciprocal effects of job conditions and the intellectuality of people's leisure-time activities. (3) Again using the data from both the men and their wives, analyzing the relationships between housework and psychological functioning. (4) Analyzing a thoroughgoing replication, conducted in Poland, of the U.S. findings on the interrelationship of social stratification, job conditions, and psychological functioning. (5) Beginning the analysis of another cross-national replication in Japan. (6) Using the data for children, assessing the processes by which the educational process affects psychological development. and (7) Assessing the effects of men's job experiences on their wives' psychological functioning and of women's job experiences on their husbands' psychological functioning.

1. Longitudinal analysis of the reciprocal effects of job conditions and psychological functioning. The intent here is a definitive assessment of whether, and if so, the extent to which, job conditions affect men's psychological functioning and whether, and if so, the extent to which, men's psychological functioning affects their job conditions. These analyses began (see the 1976-77 Annual Report) with an assessment of the reciprocal effects of one pivotal job condition--the substantive complexity of work--and one central facet of psychological functioning--the ideational component of intellectual flexibility. In that phase of the analysis, Melvin Kohn and Carmi Schooler demonstrated that the substantive complexity of work has a decided effect, essentially ongoing and continuous, on intellectual flexibility. Intellectual flexibility has a much more gradual (time-lagged), albeit in the aggregate even greater, effect on the substantive complexity of work. The causal model was then expanded (see the 1977-78 Annual Report) to a more comprehensive assessment of job structure and ideational flexibility. The substantive complexity of work proved to be the single most important occupational determinant of ideational flexibility, but several other job conditions also affect and are affected by ideational flexibility. The next step in the analysis (see the 1978-79 Annual Report) was the application of the same causal model to each of a number of other facets of psychological functioning--e.g., authoritarian conservatism, self-esteem, anxiety, and standards of morality. These analyses demonstrated the far-reaching effects of job conditions, not only on intellectual functioning, but also on self-conception and social orientation.

During the past two years, Kohn and Schooler have attempted to develop a more general causal model of job conditions and personality, a model that simultaneously assesses several job conditions and several facets of psychological functioning. The 1979-80 Annual Report summarized the difficulties resulting from some aspects of self-conception and social orientation being so highly correlated with each other and with ideational flexibility that it is not possible with ordinary statistical procedures to estimate their independent effects on other variables in the model. This problem has finally been solved. To do so, the investigators performed a "second-order" confirmatory factor analysis, based on the hypothesis that there are two principal underlying dimensions to self-conception and social orientation, self-directedness versus conformity to external authority and a sense of distress versus a sense of well-being. Self-directedness implies the beliefs that one has the personal capacity to take responsibility for one's actions and that society is so constituted as to make self-direction possible. The hypothesis that a sense of well being or of distress constitutes a second principal dimension underlying the several facets of self-conception and social orientation is based on the belief that self-directedness and conformity may each have its own psychic costs and rewards.

The second-order factor-analytic model confirmed these expectations. Self-directedness is reflected in not having authoritarian conservative beliefs, in having personally responsible standards of morality, in being trustful of others, in not being self-deprecatory, in not being conformist in one's ideas, and in not being fatalistic. Distress is reflected in anxiety, self-deprecation, lack of self-confidence, non-conformity in one's ideas, and distrust. This model, which allows over-time correlations of the residuals of the first-order concepts but deliberately does not permit any intra-time correlated residuals, fits the data well: the chi-square is 206.41, with 85 degrees of freedom, for a ratio of 2.43. Together with ideational flexibility, the model provides a partial but useful conceptualization of personality.

Treating personality in terms of three basic dimensions -- ideational flexibility, self-directedness, and distress -- enabled Kohn and Schooler to develop a general model of job conditions and some principal dimensions of personality. The causal model depicts a dynamic system in which job conditions affect all three dimensions of personality, all three dimensions of personality affect job conditions, and the three dimensions of personality affect one another. More concretely: Ideational flexibility is increased by job conditions that facilitate intellectual alertness, with substantive complexity having the strongest effect of any proximate condition of work. The main job determinant of self-directedness, too, is substantive complexity. Job conditions that sooner or later result in feelings of distress are lack of job protections, dirty work, close supervision, a low position in the supervisory hierarchy, and fewer hours of work -- all but the last suggesting oppressive working conditions, and the entire set typical of unskilled employment in the secondary labor market.

As for the effects of personality on job conditions: Ideationally more flexible men are more likely to achieve, in time, self-directed positions. A self-directed orientation results, over time, in being less closely supervised, having greater income, and doing physically lighter work, in short, in more advantageous jobs. A sense of distress results in working under greater time pressure and in a greater likelihood of being held responsible for things outside one's control. (Both of these subjective appraisals may of course be objectively inaccurate.) In time, distress leads also to doing physically lighter work.

The intrapsychic effects are impressive. Ideational flexibility both positively affects and is positively affected by self-directedness. Self-directedness both negatively affects and is negatively affected by distress. Noteworthy among these intrapsychic effects are the strong effects of self-directedness on both ideational flexibility and distress. Self-directedness affects both ideational flexibility and distress decidedly more strongly than they affect self-directedness or than they affect each other. If one of the three dimensions of personality is pivotal, it is self-directedness.

However complex the system and however diverse the effects, the findings highlight the centrality for job and personality of a mutually reinforcing triumvirate -- ideational flexibility, a self-directed orientation to self and society, and occupational self-direction. Ideational flexibility is both responsive to and productive of occupational self-direction. A self-directed orientation increases ideational flexibility and decreases a sense of distress. Occupational self-direction -- especially substantive complexity, the keystone of the job structure -- decidedly affects both ideational flexibility and a self-directed orientation.

The interrelationship of ideational flexibility, a self-directed orientation, and occupational self-direction is integral to the stratification system of the society. Occupational self-direction is substantially determined by such class-linked aspects of organizational position as ownership and hierarchical level; ideational flexibility and a self-directed orientation, in turn, affect the likelihood of an individual achieving a highly placed organizational and social position. In short, occupational self-direction, ideational flexibility, and a self-directed orientation are intertwined in a dynamic process through which the individual's place in the stratification system both affects and is affected by his personality.

2. Job conditions and the use of leisure time. The purpose of this analysis, which has been carried out by Karen Miller and Melvin Kohn, is to examine the relationship between people's job conditions and the intellectuality of their leisure-time activities. During 1979-80, Miller and Kohn expanded a measurement model of leisure-time intellectuality that they had earlier designed and developed some tentative causal models of the reciprocal effects of job conditions and leisure-time intellectuality for men. This year, they completed the analysis, refining the causal model for men and developing a comparable model for employed women.

The analysis focused on the mechanism of transfer between work and nonwork spheres of life. Several recent reviews have summarized a large but inconclusive body of research on this topic. These reviews discuss two major competing hypotheses about the nature of the relationship between work and nonwork, the so-called spillover hypothesis and the compensation hypothesis. The spillover hypothesis implies not that work is taken home or that leisure is spent in the company of coworkers, but only that there is a basic similarity in the nature of work and nonwork activities. This similarity may be interpreted as a direct process of learning-generalization by which the lessons learned in one sphere of life are carried over to other spheres of life or, alternatively, as the result of some underlying personality predisposition that affects both work and nonwork behavior -- really a distinct hypothesis. The compensation hypothesis implies a negative association between work and nonwork activities, with the causal process usually proposed being that workers

seek to compensate in their leisure for what they lack in their work (e.g., that people who do dull work seek stimulation and variety in their leisure-time pursuits). Still another hypothesis, the segmentation hypothesis asserts that work and leisure are psychologically compartmentalized, implying that no association exists between work and nonwork activities. The research literature does not indicate unequivocal support for any of these hypotheses. Nor does the research literature speak to another possibility, that some job conditions affect leisure activities simply because they provide or deplete resources necessary for those activities.

Miller and Kohn's analysis provides strong evidence for the learning-generalization hypothesis, substantial evidence for the facilitator/constraint hypothesis, equivocal evidence -- and then only for men -- for the compensation hypothesis, substantial evidence against the personality-predisposition hypothesis, and overwhelming evidence against the hypothesis that workers compartmentalize their jobs from their leisure-time activities.

A strong, positive effect of the substantive complexity of their work on the intellectuality of both men's and women's leisure-time activity is powerful evidence that people directly generalize from the job to the activities they perform in their leisure time. A small negative effect of routinization on men's leisure-time intellectuality is also consistent with the learning-generalization hypothesis, as is the positive direct effect of hierarchical position for women. The positive effects for men of leisure-time intellectuality on substantive complexity and on ownership provide some evidence that direct learning-generalization takes place also from leisure-time activities to job conditions.

The evidence is generally consistent, too, with the expectation that job conditions that provide time, energy, and money facilitate, while conditions that deplete these resources constrain, the intellectuality of leisure-time activity. Thus, job income has a modest positive effect, while long hours of work, dirtiness, and time pressure have modest negative effects on men's leisure-time intellectuality. Correspondingly, family income positively affects and long hours of work negatively affect women's leisure-time intellectuality. There is some evidence that leisure-time activities also affect these job conditions. Men's earlier leisure-time intellectuality inversely affects the number of hours worked in the current job; and women's earlier leisure-time intellectuality positively affects family income at the current time.

Two of the findings might be interpreted as providing support for the hypothesis that not only learning-generalization, but also compensation, occurs in the relationship between work and leisure, at least for men. Closeness of supervision positively affects leisure-time intellectuality. This may be a statistical artifact. If the positive relationship is real, it means that men who are closely supervised on their jobs compensate by

engaging in intellectually challenging leisure-time activities, or that men who are not closely supervised compensate for too great intellectual demands on their jobs by engaging in leisure-time activities that do not require thought. The investigators also find that heavy work increases men's leisure-time intellectuality. While this, too, might be a statistical artifact, their analyses lead them to think it is real. If so, it could be interpreted as some sort of compensation between physical and mental activity: Men who do heavy, physically demanding work may compensate by engaging in mentally demanding activities off the job that may not require physical exertion. Correspondingly, men who do sedentary work may compensate by engaging in leisure-time activities requiring heavy physical exertion, activities that often are very intellectually demanding. There is, however, another and more straightforward possible interpretation -- that physical exertion facilitates intellectual activity off the job, while lack of physical exertion on the job is detrimental to the use of intellect in leisure time.

Miller and Kohn find also that the relationships between job conditions and leisure-time intellectuality do not result solely from more intellectually flexible people selecting and being selected into more intellectually demanding jobs and leisure-time pursuits. Moreover, when they compare the findings of the reciprocal-effects causal model of job conditions and leisure-time intellectuality developed for men in this analysis with an exactly comparable model of job conditions and ideational flexibility, they find that substantive complexity has twice as great an effect on leisure-time intellectuality as on ideational flexibility. Clearly, the process of learning-generalization between activities in these two realms of life involves more than intellectual potential. There is, in addition, a carryover of patterns of behavior from each realm to the other.

3. Housework and psychological functioning. On the hypothesis that the psychological impact of the conditions of work encountered in household work would be similar to those of job conditions encountered in paid employment outside the home, Kohn and Schooler included in the 1974 follow-up survey a set of questions about the nature of the actual work performed by the respondent in taking care of the household. Insofar as possible, these questions exactly parallel those asked about work performed in paid employment. The household-work questions were asked not only of housewives, but also of working women and of men.

With this information, Carmi Schooler, Melvin Kohn, Joanne Miller, and Karen Miller have developed measurement models of the basic conditions of work encountered in housework (see the 1978-79 Annual Report) and have done multiple-regression analyses of the effects of housework on psychological functioning, particularly for women (see the 1979-80 annual report). This year, the investigators have attempted to move from multiple-regression analysis, which assumes uni-directional effects of household work on psychological functioning, to reciprocal-effects causal modelling, which allows the possibility that household work may not only affect, but may

also be affected by, psychological functioning. These analyses have proved to be even more difficult than usual, mainly because of limitations of data. Ideally, such analyses require data collected at two or more times -- a baseline measurement and a later measurement of change from the baseline. (Three measurements would be even better than two, for three measurements simplify problems of identification in causal modelling.) For the analysis of men's job conditions and psychological functioning, the data of the original 1964 survey and the 1974 follow-up survey provide just such information. For the analysis of women's job conditions and psychological functioning, there are no baseline measures of psychological functioning, but at least there is job history information, which permits simulated models. But for the analysis of women's household work and psychological functioning, there is only cross-sectional data, with no historical information about either household work or psychological functioning. For the analysis of men's household work and psychological functioning, baseline data about earlier psychological functioning do exist, but the lack of such data about household work nonetheless makes causal modelling exceedingly difficult. The analysis continues, with some hope remaining that reciprocal-effects causal modelling can be achieved. Otherwise, the investigators will have to rely principally on the multiple-regression analyses, which (as reported last year) demonstrate meaningful relationships between women's household work and psychological functioning. Similar analyses must also be done for men.

4. The Polish replication. The main purpose of this inquiry is to examine the interrelationship of social stratification, job conditions and psychological functioning in a socialist society. Specifically, the study attempts to test, in a different economic, political, and cultural context, Kohn and Schooler's central finding in their studies in the U.S., that occupational self-direction plays a crucial role in explaining the impact of social stratification on parental values and on orientations toward self and society.

Three principal co-investigators, Kazimierz Slomczynski, Krystyna Janicka, and Jadwiga Koralewicz-Zebik, carried out in 1978 in Poland a precise replication of the survey originally conducted by Kohn and Schooler in 1964 in the United States. After the data had been collected, coded, and edited in Poland, Slomczynski brought them to NIH, where he, Joanne Miller, and Melvin Kohn have been analyzing them. The 1978-79 Annual Report reviewed their efforts to develop methods that assure cross-national comparability of indices. In 1979-80, the main thrust of the work was addressed to one of the central questions of the Polish replication: Do people's positions in the system of social stratification bear the same relationships to their values and orientations in socialist Poland as in the capitalist U.S.? In general, the investigators found the relationships of social stratification position with values and with social orientation to be remarkably similar in Poland and the United States. But, for reasons they still do not fully understand, the relationships between social stratificational position and self-conception are somewhat different in the

two countries. In particular, in the U.S., higher social position is associated with more self-confidence (r= 0.22), but in Poland the opposite is true (the corresponding correlation being -0.12)

During the past year, the research has been addressed to ascertaining whether job conditions play as crucial a role in explaining the effects of the two principal components of social stratification position -- i.e. occupational position and education -- on values and orientations in Poland as in the United States. The investigators found, for both the United States and Poland, that the effects of occupational position on parental values and on social orientations are substantially attributable to occupational self-direction. The situation, though, is different for self-conceptions. In the United States, occupational self-direction largely explains the effects of occupational position on two pivotal facets of self-conception -- self-confidence and self-deprecation; in Poland, occupational self-direction has no such explanatory power.

Similarly for the role of occupational self-direction in explaining the impact of education on values and orientations: For the United States, occupational self-direction accounts for a substantial portion of education's effects on parental values and on social orientations. For Poland, occupational self-direction accounts for even more of current education's effects on values and on social orientations. Self-conceptions again provide the one sharp contrast between the U.S. and Poland. In the U.S., occupational self-direction largely explains the impact of education on self-conception. In Poland, occupational self-direction explains little or none of the effect of education on any aspect of self-conception except idea-conformity.

In short, in Poland even more than in the United States, the effects of social-stratification position on parental valuation of self-direction and on social orientations result substantially from men of higher social-stratification position exercising greater self-direction in their work. But, although occupational self-direction similarly accounts for much of the relationship of social-stratification position with self-conception in the U.S., it fails to do so in Poland.

The study thus confirms, for both Poland and the United States, that higher social-stratification position is associated with valuing self-direction and with holding social orientations consonant with valuing self-direction -- namely, a nonauthoritarian, open-minded orientation, personally responsible standards of morality, trustfulness, and receptiveness to change. An interpretation of these associations need not, indeed should not, focus on anything unique to the United States or to Poland. An adequate interpretation must transcend the two countries, which means, inter alia, that it must apply equally well in both capitalist and socialist society. A more general explanation of the psychological ramifications of social stratification in industrialized society is readily found in Kohn and Schooler's original interpretation of the U.S. data --

that social stratification is associated with values and with social orientations in large part because men of higher position have greater opportunity to be self-directed in their work, i.e., to work at substantively complex tasks, free of close supervision, and not subject to routinization.

The present study not only confirms the findings that underlie this interpretation, both in the United States and in Poland, but also reformulates and tests parts of the interpretation for which Kohn and Schooler advanced only an a priori argument. Thus, this study reconceptualizes and empirically assesses the reciprocal relationship between social-stratification position and occupational self-direction. It reassesses the extent to which the effects of social stratification on values and orientations are indirect, via occupational self-direction. It also demonstrates that occupational self-direction has an actual causal impact on values and social orientations. By so doing, it provides solid evidence for the interpretation that self-direction in one's work leads to valuing self-direction and to having a more open, flexible orientation to society. Lack of opportunity for self-direction in one's work leads one to value conformity to external authority and to view social reality as hostile and threatening. In short, the lessons of the job are generalized to one's stance toward the larger society, in socialist as well as in capitalist society. This does not mean that these processes are necessarily the same in all socialist and all capitalist societies, but it certainly does mean that the original U.S. findings are not restricted to capitalist countries.

The one noteworthy difference between Poland and the United States is in the relationship between social-stratification position and self-conception, in particular, self-confidence. Although the associations between social stratification and self-conceptions in the United States are of only modest magnitude, the pattern is consistent with those for values and social orientations -- men of higher social position have more favorable conceptions of self. As with values and social orientations, the relationships between social stratification and self-conceptions result largely from men of higher position being more self-directed in their work. In Poland, there is not the same association between social stratification and self-conceptions; in fact, men of higher social position are somewhat less self-confident and more anxious than are men of lower social position. Nor does occupational self-direction have much impact on self-conceptions. Somehow, in the United States, the learning-generalization process by which the lessons of the job are carried over to one's values and social orientations occurs also (albeit, not as strongly) for one's orientations to self. In Poland, either occupational self-direction does not enhance self-conception or something interferes with such enhanced self-conception being carried over to non-occupational spheres of life. We do not know which.

5. The Japanese replication. Another major replication of the Kohn-Schooler survey has been conducted in Japan by Atsushi Naoi and Ken'ichi Tominaga of the Department of Sociology of Tokyo University. The replication involved interviewing a probability sample of more than 800 employed Japanese men, using a questionnaire containing all the questions necessary for indexing job conditions and those aspects of psychological functioning measured in the American and Polish studies. This replication is of particular interest not only for the cross-cultural comparisons it will provide, but also because the structure of industry is rather different in Japan, with a much sharper cleavage between the primary and secondary labor markets than is true in the United States.

Data collection took place during the summer and fall of 1979 and the data were coded for statistical analysis in Japan during the 1979-1980 academic year. Data-analysis began in October, 1980, when Naoi came to the Laboratory as a Visiting Scientist to work colloboratively with Carmi Schooler. During this past year, measurement models were developed for occupational self-direction, intellectual flexibility, and several facets of self-conception and social orientation (anxiety, self-confidence, self-deprecation, authoritarian-conservatism, morality, fatalism, acceptance of change and idea conformity). These measurement models are generally similar to those that had previously been developed in analyses of the American and Polish data.

Preliminary models of the causal relationship between job conditions and ideational flexibility have also been tested. The initial results indicate that both occupational self-direction and bureaucratization have positive effects on ideational flexibilty. Further analysis is presently being carried out, both of this model and of models examining the causal interrelationships between job conditions and other psychological variables.

6. Educational experience and children's psychological development. The purpose of Karen Miller, Melvin Kohn, and Carmi Schooler's analysis is to examine the processes by which students' educational experiences, particularly the degree of self-direction they are able to exercise in their educational endeavors, affect their psychological functioning. In their analyses of social stratification and psychological functioning, Kohn and Schooler long ago found that education has important independent effects on psychological functioning -- greater than those of occupational position, for example. They did not at that time have data to study the processes by which education exerts these psychological effects. Relevant data were subsequently collected in the 1974 follow-up survey as part of the interview with children of the primary respondents. The interview schedule for these "children" -- now aged 13 to 25 -- contains an intensive battery of questions about the current educational experiences of all those respondents still in school. These questions are designed to parallel those previously found to be potent for analyzing occupational experience, focusing on such dimensions of the educational experience as its

substantive complexity, how closely it is supervised, time-pressure, and the like. The intent is to see whether the concepts and methods developed for the study of occupational experience can be applied as well, and with similar results, to the study of educational experience. In effect, Kohn and Schooler have developed a theory of occupation as an educational experience. Now Miller, Kohn, and Schooler are attempting to apply the same concepts to schooling itself.

The main thrust of the analysis thus far has been to develop a comprehensive measurement model of a concept central to the study: "educational self-direction." This is an overarching concept that encompasses the substantive complexity of school work, its routinization, closeness of supervision by the teacher, and the elective nature of the coursework. The first three are analagous to the three aspects of occupational self-direction developed in analyses of job conditions: substantive complexity, routinization, and closeness of supervision. Preliminary analyses show that these aspects of self-direction relate together in the educational context in ways that are similar to how they interrelate on the job. The model of educational self-direction also included electivity, a variable that is important specifically for schoolwork. As expected, it is positively related to the substantive complexity of schoolwork and negatively related to closeness of supervision and routinization of schoolwork.

The investigators are in process of developing measurement models of intellectual flexibility for students, the next step in a systematic analysis of the educational process and its relationship to psychological functioning.

7. The effects of men's job experiences on their wives' psychological functioning and of women's job experiences on their husbands' psychological functioning. Carmi Schooler, Melvin Kohn, and Ronald Schoenberg are working on what is undoubtedly one of the most complex analyses of the entire occupations study. The intent is to determine the extent to which, and the processes by which, a married person's job experiences affect his spouse's values, self-conceptions, social orientations, and intellectual functioning, taking into account the spouse's own job experiences and social characteristics. There is some evidence (from Pearlin and Kohn's analyses of the Turin study some years ago) that a man's job experiences affect his wife's values, but the evidence is limited and based on rather primitive statistical methods. The present investigators intend a much more comprehensive analysis, not only of the effects of men's job conditions on their wives' values, but also of each spouse's job conditions on many facets of his partner's psychological functioning, using complex causal models. The inquiry is still at a very early stage--attempting to develop causal models that appropriately combine the longitudinal data available for men with the cross-sectional data available for women.

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Kohn, M. L.: Personality, Occupation, and Social Stratification: A Frame of Reference. In Donald J. Treiman and Robert V. Robinson (Eds.): Research in Social Stratification and Mobility. New York: JAI Press, Vol. 1, 1981, pp. 267-297.

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Miller, J.: Individual and Occupational Determinants of Job Satisfaction: A Focus on Gender Differences. Sociology of Work and Occupations. Soc. Work Occup. 7: 337-366, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 MH 00674-11

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Social, Psychological, and Biological Interactions in Schizophrenics and Normals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Schooler	Research Psychologist	LSES	NIMH
Other:	T. Zahn	Research Psychologist	LP	NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology, NIMH

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This year saw the completion of the linear structural equations analysis of the results of a carefully planned, controlled and measured socially oriented ward treatment program in a Veterans Administration Hospital. The results indicate that in an experimental ward program for chronic schizophrenics the general level of activity, much of it social, was disruptive to the psychological functioning of patients, particularly sicker ones. Anti-psychotic drugs positively affected psychological functioning and also decreased social behavior.

PHS-6040
(Rev. 10-76)

This year saw the completion of the linear structural equations analysis of the results of a carefully planned, controlled and measured socially oriented ward treatment program in a Veterans Administration Hospital. The results indicate that in an experimental ward program for chronic schizophrenics the general level of activity, much of it social, was disruptive to the psychological functioning of patients, particularly sicker ones. Anti-psychotic drugs positively affected psychological functioning and also decreased social behavior.

A review of other studies indicates that we are not unique in finding that intensive socio-environmental therapies have adverse effects on schizophrenics. Furthermore, a wide range of studies indicates that schizophrenics are prone to avoid social interaction and show a decrement in functioning as the intensity of such interactions increases.

Unfortunately, although various hypotheses seem feasible, little is known about the reasons for schizophrenics' social dysfunction and little research is presently being done. Given the recent findings that phenothiazine treatment by itself is insufficient to keep many patients out of the hospital and that those who stay in the community show an increase in withdrawal, the development of successful treatment programs for schizophrenia may hinge upon our learning more about the nature of and reasons for the schizophrenic's social dysfunction.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00679-01 LSES

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Structural Equation Models in the Analysis of Data with Measurement Error

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI :	Ronald J. Schoenberg	Research Sociologist	LSES	NIMH
OTHER:	Joanne Miller	Research Sociologist	LSES	NIMH
	Kazimierz M. Slomczynski	Visiting Scientist	LSES	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this work is to further develop the methods and techniques for the specification and estimation of the parameters of structural equation models of survey data that contain random and nonrandom measurement error. Included in this are methods for the identification of the models, estimation of the means of unobserved variables, the determination of model condition, and the cross-national comparison of structural models (specifically, the United States and Poland).

PHS-6040
(Rev. 10-76)

Project Description:

Over this last year the ability of the investigators in this Laboratory to explore larger and more complex models was considerably enhanced. The key element of the array of statistical procedures maintained by Ronald Schoenberg, the person responsible for the development and maintenance of these procedures, is a computer program that estimates the parameters of complex structural equation models that include measurement error terms, using a sophisticated method for optimizing the maximum likelihood function, and which produces a variety of diagnostic material that pinpoints weaknesses in specification and provides information for re-specification. First of all, certain efficiencies were introduced in the program that allowed models with over 300 open parameters to be estimated (nearly twice the previous size) while cutting in half the amount of computer time required for the estimation, in effect a quadrupling of productivity. Second, a new feature was added to the program that improves the evaluation and diagnosis of models and which will have important consequences for the analysis of structural models in general. This technique has been written up in a paper to be presented at the American Sociological Meetings in Toronto. A major difficulty in the estimation of large complex structural models is the determination of their identification. Very simply, identification is the confirmation that the estimated parameters are unique (if not then there will be an infinite number of possible estimates severely compromising the findings). The new technique determines the identification of each open parameter giving a confidence to findings that was not available before. Moreover, the concept of identification of parameters is extended to incorporate the idea of "near-identification" so that marginally identified parameters can be pinpointed. "Condition" numbers are calculated that measure the degree of identification and permit a parameter by parameter evaluation of the model. A bad "condition" number for a particular parameter indicates that the model is perhaps asking too much of the data and that perhaps the model should be further constrained. And because the diagnostic information is so specific it is possible to more directly determine the remedy.

Finally, a completely revised and streamlined version of this program, called MILS (Multiple Indicator Linear Structural models), was made available for general use. Several universities and research organizations had expressed interest and were sent copies.

Also, several of the advances in statistical methods pioneered at this Laboratory were reported in papers that have been submitted for publication. One paper describes the procedure for calculating standardized first order partials of the maximum likelihood function with respect to the fixed parameters of the model. This allows the evaluation of the plausibility of the constraints made on the model. If a constraint (a parameter fixed to zero, for example) sufficiently conflicts with the data then the first order partial with respect to that parameter will be large, suggesting that it should be opened. Another paper discusses the estimation of factor means and their standard deviations. This issue has been misconceived heretofore, in the opinion of the author, and this paper

proposes to clear away some of the confusion. And finally, the most important contribution to the analysis of structural models in general is presented in the paper mentioned above on identification. This paper has already attracted the attention of methodologists and the session at the Annual Meetings wherein it is presented promises to be well-attended.

Ronald Schoenberg has in addition been responsible for a weekly lecture on statistical methods which has considerably upgraded the methodological proficiency and expertise of the members of the lab. During the year a standard text, Econometric Methods by J. Johnston, was covered chapter by chapter. Detailed coverage of the factor analysis model, measurement models, and their relation to structural models came after that. Other topics were matrix algebra, statistical inference, and discussions of the interpretation of structural models.

Some methodological issues with regard to the comparison of measurement models across populations were dealt with in a paper by Joanne Miller, Kazimierz Slomczynski, and Ronald Schoenberg. Procedures for the comparison of measurement models are not fully worked out in the comparative literature and this paper will constitute an original contribution toward such methods. First, a series of nested models is proposed that includes indicators of the concept in a certain order. Then a chi-square test is used to determine a stopping point. The indicators that are left, called the core indicators, are demonstrated, then, to be structurally identical in the two populations. In the data presented in the paper the authors demonstrated that authoritarian conservatism is measured consistently by five core items in Poland and the United States. This is actually a remarkable finding which was made possible by the new methods and which are the more remarkable for the fact that the methods used are more rigorous than any previous methods used in the comparative literature.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00851-17 LBEB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Brain Mechanisms of Species-typical Behavior in Squirrel Monkey (Saimiri sciureus)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul D. MacLean	Chief	LBEB NIMH
Other:	Robert E. Gelhard	Biologist	LBEB NIMH
	Thalia K. Bussard	Biologist	LBEB NIMH
	Carroll R. Harbaugh	Biol. Lab. Tech.	LBEB NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	0.4	1.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with an investigation of brain mechanisms involved in species-typical prosematic (rudimentary signaling) communication. Observations are made on the effects of brain lesions on a highly predictable mirror display of one variety of squirrel monkeys (Saimiri sciureus). In an attempt to differentiate tegmental structures crucial for the display, a means is being sought for selectively destroying cellular elements without damage to fibers of passage. The present report describes the effects of applying puromycin and deferoxamine mesylate (a chelating agent for iron) at sites in the tegmentum.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This long-term project is concerned with an investigation of brain mechanisms involved in species-typical prosematic (rudimentary signaling) communication. The work has dealt specifically with the effects of brain lesions on the highly predictable mirror display of one variety of squirrel monkeys (see M-NP-LI-17, 1964). The results indicate that the forebrain structures most crucial for the display are located in the striatal complex, a group of ganglia and surrounding gray matter that are a fundamental part of the forebrain in reptiles, birds, and mammals. The analysis has shown that the medial segment of the globus pallidus is a region of convergence of neural mechanisms essential for the expression of the mirror display. Electrocoagulation of this region or of its projecting pathways to the ventral thalamus and tegmentum may eliminate or fragmentize the display. Tegmental structures appear to play a more critical role than the ventral thalamus. In making the next step to differentiate the tegmental structures most crucial for the display, it is desirable to devise a means of destroying the cellular elements of tegmental nuclei without damage to fibers of passage. The present phase of the work deals with this problem.

Methods Employed: The experiments primarily involved two chemicals--(1) puromycin and (2) deferoxamine mesylate (USP). Based on experiments on rats, puromycin has been recommended as an agent for the selective destruction of nerve cells without damage to fibers of passage. Deferoxamine is a chelating agent for iron; an assay on its local effects on nerve cells and fibers was performed apropos of the high iron content of pars reticulata of the substantia nigra and strionigral fibers. Two methods were used: (1) a microinjection technique and (2) a method described by one of us (PD MacL, 1957) for depositing measured amounts of solid substances. In each instance, the "chemode" was fixed in place as a preventative against reflux of the chemical along the chemode track. Two squirrel monkeys were used for these experiments.

Major Findings:

Puromycin. Microinjections of puromycin (and actinomycin) in the amount and dose calculated to correspond to that recommended for rats (0.05 μg in 1.0 μl) failed to result in damage significantly beyond that caused by the "chemode." Deposits of puromycin powder in amounts up to 0.06 mm^3 likewise failed to produce surrounding changes sufficiently large for the purposes of the present study. In some cases healthy appearing nerve cells were found within 0.5 mm of the tip of the chemode. At one site in the globus pallidus there was a sphere-shaped paling of the nervous tissue extending 1.25 mm from the tip of the chemode. Both cells and fibers were damaged. Since there was paling of the striopallidal fibers in Wilson's pencils, there are indications that the diffusing substance affects fibers of passage as well as cells.

Deferoxamine. Deferoxamine deposited in solid form in amounts of 0.4 mm^3 did not result in the disappearance of cells or injury of fibers

in either the globus pallidus or substantia nigra. In the case of a nigral deposit, the large cells of the neighboring red nucleus showed a marked reduction in number. In counts of these cells made on 7 evenly spaced sections, there was a reduction of 83 percent as compared with the normal appearing cell group on the contralateral side. These changes (in addition to symmetrical loss of tissue in the basal nucleus of the amygdala, N. centrum medianum, and lateral geniculate bodies) call for further experimentation.

Significance to Biomedical Research and the Program of the Institute: A long-time obstacle to progress in neurobehavioral work has been the inability to produce lesions that will selectively destroy cellular areas and spare fibers of passage. The preliminary finding that deferoxamine practically eliminates the large celled part of the red nucleus holds promise for testing the behavioral effects of eliminating this portion of the midbrain tegmentum.

Proposed course. In view of the equivocal outcome of the experiments just described, we will continue the experimental work involving electrocoagulative lesions in the tegmentum. The next targeted structures are the deep tegmental nuclei of Gudden, the dorsal raphe nuclei, and the superior central nucleus of Bechterev.

Publications:

MacLean, P.D.: Role of transhypothalamic pathways in social communication. In Morgane, P.J., and Panksepp, J. (Ed.): Handbook of the Hypothalamus, Vol. 3--Part B, Behavioral Studies of the Hypothalamus. New York and Basel, Marcel Dekker, Inc., 1980, pp. 259-287.

MacLean, P.D.: Family feeling in the triune brain. Psychology Today 15: 100, 1981.

MacLean, P.D.: On the origin and progressive evolution of the triune brain. In Armstrong, E., and Falk, D. (Eds.): Primate Brain Evolution: Methods and Concepts. New York, Plenum Press (in press).

Murphy, M.R., MacLean, P.D., and Hamilton, S.C.: Species-typical behavior of hamsters deprived from birth of neocortex. Science 213: 459-461, 1981.

Radna, R.J., and MacLean, P.D.: Vagal elicitation of respiratory-type and other unit responses in striopallidum of squirrel monkeys. Brain Res. 213: 29-44, 1981.

Radna, R.J., and MacLean, P.D.: Vagal elicitation of respiratory-type and other unit responses in basal limbic structures of squirrel monkeys. Brain Res. 213: 45-61, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00787-02 LBEB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pilot Experiments on Role of Rostral Limbic Cortex in (1) Species-typical Vocalizations and (2) Ludic Behavior of Squirrel Monkeys (Saimiri sciureus).

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul D. MacLean	Chief	LBEB NIMH
	John D. Newman	Physiologist	LDN NICHD
Other:	Carroll R. Harbaugh	Biol. Lab. Tech.	LBEB NIMH
	Robert E. Gelhard	Biologist	LBEB NIMH
	Thalia K. Bussard	Biologist	LBEB NIMH

COOPERATING UNITS (if any)

J.D. Newman, LDN, NICHD

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pilot experiments are being performed on the effects of extensive ablations of the rostral limbic cortex on (1) the isolation call and (2) ludic behavior of adolescent squirrel monkeys. The present phase of the work is concerned with devising a satisfactory surgical procedure.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: In a preceding project (Z01 MH 00787-01 LBEB, 1980) it was found that lesions of certain tegmental structures either eliminated or markedly altered the species-typical isolation call of squirrel monkeys. On the basis of stimulation studies it has been reported that the rostral limbic cortex (posterior gyrus rectus, subcallosal gyrus, pregenual and anterior cingulate cortex) may be implicated in the production of the isolation call, as well as other species-typical vocalizations of the squirrel monkey. (1) As a deciding factor in planning further experimentation, it is the purpose of the present project to learn whether or not extensive destruction of the rostral limbic cortex will affect species-typical vocalizations. (2) In addition, advantage will be taken of the present experiments to learn whether or not the intended lesions will affect ludic behavior of squirrel monkeys. As reported in a preceding project (Z01 MH 00874-03 LBEB), neonatal ablation of the cingulate cortex and underlying hippocampal formation of hamsters appears to interfere with the expression of play-fighting.

Methods Employed: Experiments are performed on adolescent male and female squirrel monkeys.

Recording of vocalizations. Isolation calls are recorded with Uher microphone and tape recorder while subjects are isolated for 30 minutes. A VII model 700 sound spectrograph is used for spectrographic analysis. In addition to the isolation call, attempts will be made to record vocalizations of the mirror display.

Observations on ludic behavior. The ludic behavior of adolescent squirrel monkeys includes (1) chase; (2) tail pulling; (3) pulling on head and ears; (4) wrestling; (5) sham biting; and (6) quasi-copulatory activity with intervening genital displays.

Surgical procedures. In a preceding project (M-NP-LI-23, 1971), it was found that the horizontal insertion of a 4 mm wide leucotome through the septum and into the anterior thalamus resulted in a remarkably symmetrical infarction of the entire septum, the posterior part of the medial frontal cortex, the most rostral part of the cingulate gyrus, and the ventral part of the supracallosal cingulate gyrus as far caudally as the splenium. The lesions were interpreted as being attributable to occlusion of the anterior cerebral artery distal to the point at which it becomes a single vessel. In the initial experiments, an attempt has been made to learn whether or not occlusion of the anterior cerebral above the level of the septum would spare the septum, but result in symmetrical lesions of the medial frontal cortex and cingulate gyrus.

Major Findings: Experiment 1. In the first experiment, surgery was performed on an adolescent male squirrel monkey with preoperative documentation of its play behavior. After turning a left frontal bone flap, reflecting the dura, and cauterizing a bridging vein at AP 14, the

left frontal lobe was gently retracted from the midline by the insertion of cotton patties, giving an excellent exposure of the anterior cerebral artery. In confirmation of a finding in a single case cited in a preceding report (M-NP-LI-36, 1968) cauterization of the anterior cerebral below the knee of the corpus callosum did not occlude its distal ramifications, thus indicating an extensive collateral blood supply. The vessel was then cauterized just above the knee of the corpus callosum. It continued to show filling caudal to the occlusion. Surgery was then terminated.

On the next day the animal was in excellent condition, feeding and moving rapidly about its cage. The monkey was avocal, however, and no isolation calls were recorded until the 16th postoperative day. No signs of play appeared until the 49th postoperative day.

The examination of serial sections of the brain revealed a small infarct of the knee of the corpus callosum and the overlying cingulate gyrus on the left side. A small infarction extended into the medial wall of the left frontal lobe.

Experiment 2. Because of the failure to obtain occlusion of the anterior cerebral in the above experiment, an expendable monkey was used for observing the effects of occlusion of the stem vessels of this artery. Here again there was continued filling of the anterior cerebral after cauterization of the two stem vessels, indicating an extensive collateral blood supply. The anterior cerebral was then resected up to the point at AP 9 where it divides into two vessels. This procedure resulted in infarction of the preoptic region and entire septum, together with the rostral limbic cortex extending from the posterior part of the gyrus rectus to the anterior cingulate cortex above the septum. There was also infarction of the intermediate parts of the caudate nucleus.

Significance to Biomedical Research and the Program of the Institute: In the evolution from reptiles to mammals, two major behavioral developments were vocal communication and ludic behavior. The isolation call, serving to maintain maternal-offspring contact and contact of members of a group, probably represents one of the most basic mammalian vocalizations. One of us has proposed that the development of play behavior may have been conducive to harmony in the nest and social affiliation later on. Since play is not observed in reptiles, and the same would be largely true of vocalization and parental behavior, it is of interest that a major subdivision of the limbic system represented by the anterior thalamic nuclei and its projections to the cingulate gyrus has no apparent counterpart in the brains of existing reptiles. This system is closely allied to another subdivision of limbic cortex for which the septum is an internode. The transient elimination of the isolation call and play behavior in the first pilot experiment of the present study in which there was a small and predominantly unilateral lesion of the rostral limbic cortex merits a continuation of these experiments.

Proposed Course: To be continued.

Publications:

Newman, J.D., and MacLean, P.D.: Effects of tegmental lesions on the isolation call of squirrel monkeys. Brain Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00788-02 LBEB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Role of Paleostriatum in Species-typical Behavior of Chickens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ann A. Hagan	Staff Fellow	LBEB, NIMH
	James L. Hill	Biologist	LBEB, NIMH
	P.D. MacLean	Chief	LBEB, NIMH
Other:	Robert E. Gelhard	Biologist	LBEB, NIMH
	Thalia K. Bussard	Biologist	LBEB, NIMH
	Carroll R. Harbaugh	Biol. Lab. Tech.	LBEB, NIMH

COOPERATING UNITS (if any)

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NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.825	0.6	0.25

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present report deals with one of a series of comparative studies on the functions of the striatal complex, a basic part of the forebrain in reptiles, birds, and mammals. An attempt is being made at surgical or pharmacological elimination of telencephalic structures dorsal to the paleostriatum in chickens (Gallus gallus) in order to assess what forms of behavior are retained or eliminated in such preparations.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: A primary aim of this Laboratory is to investigate the functions of the striatal complex which constitutes a basic part of the forebrain in reptiles, birds, and mammals. In a preceding project (Z01 MH 00870-03 LBEB), it was shown by neonatal telencephalic ablations in hamsters that structures comprising the striatal complex and limbic system were sufficient for the expression of most forms of species-typical behavior. If there was an additional loss of the midline limbic cortex, such animals failed to develop play-fighting and there were deficits in maternal behavior. In reptiles and birds, the paleostriatum lying ventral to the dorsal medullary lamina represents the greater part of the striatal complex. In birds, intracleidoic surgery or administration of a nucleic acid alkylating agent presents the possibility of eliminating most of the telencephalon above the paleostriatum and then observing what species-typical forms of behavior are either retained or eliminated. (It has long been known that birds deprived of the entire telencephalon are unable to engage in spontaneous, directed activities.) The outcome of the surgical and chemical procedures will be described, respectively, under the captions EXPERIMENT I and EXPERIMENT II.

EXPERIMENT I

Methods Employed:

Behavioral observations. A complete behavioral inventory is required for the kind of experiments in question (see Z01 MH 00789-01 LBEB). For the preliminary experiments, observations were limited to the two weeks following hatching when the behavioral items of main interest fall into the four following categories: (i) locomotion (walking, running, jumping, hopping); (ii) feeding (ground-pecking, drinking, scratching, head-shaking); (iii) vocalizations (twitters, isolation peeps); and (iv) assorted activities (stretching, preening, fear-crouch posturing).

Ablation techniques. Intracleidoic ablation of the structures above the dorsal medullary lamina was attempted by microsurgical aspiration or by electrocoagulation. After two weeks the birds were sacrificed and the brains removed for examination.

Major Findings: Of the chick embryos subjected to surgery between 6 and 11 days of incubation, none survived until hatching.

Ten chicks in which surgery had been performed on the 19th or 20th day of incubation hatched and developed normally. All proved to have superficial ablations of the telencephalon. Microscopic examination of the brain with the most extensive lesions showed partial ablation of the dorsal ventricular ridge of the hemisphere extending downwards into hyperstriatum ventrale. There was only superficial damage to the right hemisphere.

EXPERIMENT II

Because of failure to achieve the desired result by surgery, an attempt was made to prevent the development of the dorsal ventricular ridge by the intracleidoic administration of methlazoxymethanol (MAM) (a cytotoxic agent that produces necrosis of dividing nerve cells) at critical times in neurogenesis.

Methods Employed:

Behavioral observations. As part of this pilot study, a special effort was made to devise tests for quantifying behavior of hatchlings. Of several tests (including open field, visual cliff, and pecking) the one involving the recording of the isolation peep proved to be the most reliable and accurate.

Administration of MAM. It has been reported that most of the cells forming DVR are generated on days 5-9 of incubation. In the present experiments, MAM was dropped onto the chorioallantoic membrane in various doses on particular days between the 4th and 10th day of incubation. It was given in amounts of 3, 6, 12, or 18 drops (about 0.3 mg per drop).

Major Findings: Aside from intact controls, 89 eggs were treated with MAM. Eleven subjects survived until the time of sacrifice, 14-19 days after hatching. Table 1 lists the brain weights for 3 controls and 6 MAM-treated animals for which measurements are available. In contrast to reported findings on rodents, MAM (with the doses used in the present experiments), produced no conspicuous micrencephalay. Although the histological examination of the brains is incomplete, findings on representative animals showed no notable differences between the development of structures above and below the dorsal medullary lamina.

There were no notable behavioral differences between the control and MAM-treated birds. An incomplete analysis of the sonograms of the isolation peeps indicates some structural differences in the initial part of the vocalization in the MAM-treated birds.

Significance to Biomedical Research and the Program of the Institute: If a means can be found to produce an avian preparation that will survive with only the paleostriatal portion of the telencephalon, it provides a valuable research opportunity for delving into special questions relevant to behavior, neuroanatomy, and neurochemistry.

Proposed Course: To be continued. In the next experiments, MAM will be administered by intravascular injection.

Publcations: None.

Table 1. Brain weights and measurements for MAM-treated and control chicks, aged 19 days at time of sacrifice.

# drops MAM*		incubation Day	total brain weight (gm)	Caliper Measurements for Cerebrum width (cm)	length (cm)	height (cm)
0			1.89	1.66	1.20	0.97
0			1.73	1.67	1.20	0.86
0	-		1.71	1.68	1.17	0.90
	X± SD:		1.78 ± 0.08	1.67±0.01	1.194±0.01	0.91±0.06
3		8	1.69	1.62	1.17	0.01
3		10	1.70	1.70	1.15	0.99
3		10	1.70	1.69	1.18	0.92
3		10	1.60	1.65	1.17	0.91
6		9	1.57	1.64	1.13	0.98
12	-	9	1.46	1.58	1.13	0.92
	X± SD:		1.57 ± 0.11	1.65±0.04	1.15±0.02	0.95±0.04

*0.3 mg per drop

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00791-01 LBEB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Neurogenesis in the Avian Telencephalon

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ann A. Hagan	Staff Fellow	LBEB, NIMH
Other:	Thalia K. Bussard	Biologist	LBEB, NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.2	0.05

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In connection with a behavioral study on the functions of the paleostriatum an attempt is being made to prevent the development of the overlying dorsal ventrical ridge by the intracleidoic application of methylazoxymethanol (MAM, a nucleic acid alkylating agent) in chickens (Gallus gallus). For this investigation, information is needed regarding the effect of MAM at critical times in neurogenesis. A special protocol involving the combined use of MAM and ^{3}H-thymidine is being used for this purpose. In addition, a study of neurogenesis in the Pekin duck (Anas platyrhynchos) has been initiated.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: In connection with a behavioral study described in Project No. Z01 MH 00788-02 LBEB, an attempt is being made to prevent the development of the dorsal ventricular ridge (DVR) of the chicken (Gallus gallus) by means of the cytotoxic agent methylazoxymethanol (MAM). For this purpose, information is needed regarding the optimum time for destroying developing nerve cells that make up the structures of DVR. At present, there exists only one autoradiographic study on histogenesis in the avian telencephalon. More information is needed not only in regard to temporal appearance, but also the migratory movements, of cells. The possibility suggests itself that the combined use of of MAM and tritiated thymidine (^{3}H-T) as a marker might provide a more complete picture than could be obtained with the latter agent alone. For example, the administration of MAM on one day, followed by the injection of ^{3}H-T on the next day (combined with a reverse order of administration for another embryo of the same age) might, reveal the absence or displacement of cells dividing or migrating, respectively, at the time of the injection of MAM. The present pilot study deals with this problem. In addition, an autoradiographic study employing ^{3}H-thymidine alone is being performed on the Pekin duck (Anas platyrhynchos). The longer hatching time for this animal (28 days) might provide more leeway with respect to critical times for injecting MAM.

Methods Employed: Chicken eggs are injected with MAM and with 20 Ci ^{3}H-T according to the schedule shown in Table 1. After windowing the shell, solutions of these agents are dropped onto the exposed chorioallantoic membrane. The volume of the injection in each case is about 0.2 cc (3 drops), amounting to a dose of 1.7 mg for MAM and 20 Ci for ^{3}H-T. The embryos are sacrificed on days 16-17.

Table 2 gives the schedule for injecting the duck eggs with ^{3}H-T, together with the day of sacrifice. The same amount of marker is used as for the chicken eggs.

Histological preparation. After sacrifice, the animals are perfused through the heart with 0.9% saline and 10% formalin. The brains are embedded in paraffin and sectioned at 6 . Sections are mounted on slides, dipped in Kodak nuclear track emulsion NTB2, exposed for 2-4 weeks, and developed for autoradiography.

Major Findings: The brains are now being prepared for histological examination.

Significance to Biomedical Research and the Program of the Institute: In addition to the practical applications for the behavioral study mentioned above, the present study should provide additional information about neurogenesis in the avian telencephalon. Such knowledge may help to give give insights into the marked divergence in the evolution of the brain in birds and mammals.

Proposed Course: To be completed.

Publications: None

TABLE 1. Schedule for chick egg injections with methylazoxymethanol (MAM) and ^{3}H-thymidine (^{3}H-T)

	Day(s) of Injection and Number (#) of Survivors						
MAM/^{3}H-T Survivors	4/5 (3)	5/6 (3)	6/7 (3)	7/8 (3)	8/9 (3)	9/10 (3)	
^{3}H-T/MAM Survivors	5/4 (3)	6/5 (5)	7/6 (3)	8/7 (3)	9/8 (3)	10/9 (3)	
^{3}H-T Survivors	4 (2)	5 (3)	6 (3)	7 (4)	8 (4)	9 (2)	10

TABLE 2. Schedule for duck egg injections with ^{3}H-thymidine

incubation day for injection	Incubation day for sacrifice					
	6	8/9	12/13	16/17	20/21	23/24
4	1	1	2	2	2	-
8	-	/1	3	2	2	2
10	-	-	-	-	2	-
12	-	-	/2	3	3	2
14	-	-	-	-	1	-
16	-	-	-	/2	2	2
20	-	-	-	-	/2	2
23	-	-	-	-	-	/2

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00792-01 LBEB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Pinealectomy on the Ontogeny of Thermoregulation in the Chick

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ann A. Hagan	Staff Fellow	LBEB, NIMH
	E. Carol Roth	Staff Fellow	LBEB, NIMH
	Jan J. Roth	Staff Fellow	LBEB, NIMH
Other:	Thalia K. Bussard	Biologist	LBEB, NIMH

COOPERATING UNITS (if any)

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Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.65	0.5	0.15

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pineal glands in 5 Gallus gallus chicks were removed just prior to hatching. The ability of pinealectomized chicks to defend body temperature during a cold challenge was compared with sham-operated and control chicks. The ontogeny of thermal competency in all three groups during the three weeks after hatching is being examined.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The pineal gland has been shown to be involved in the complex process of temperature regulation. In this connection, previous investigations have not addressed the question as to whether or not the pineal serves to regulate metabolic excesses in cold environments, a possibility that might help to explain the large size of the pineal gland in some polar animals (e.g., lemming, weddel seal, sealion, walrus). In this present study we have tested the "cold stress" hypothesis in the young domestic chicken, which is essentially ectothermic upon hatching and which develops precise endothermy as the animal matures, and acquires feather insulation.

Methods Employed: Fifteen baby chicks (Gallus gallus) were employed in this study. Five were pinealectomized 1-2 days prior to hatching; five received sham operations; five were control animals. The chicks were tested by decreasing the temperature (35°C to 15-10°C) after the birds were placed for 10-20 minutes (in their thermoneutral zone) in a controlled-temperature chamber. Cloacal temperatures were recorded continuously on a Honeywell multipoint temperature recorder accurate to 0.1°C. The experiments were conducted near midday over a 3-week period. By that time the control chicks had become precise endotherms and resistent to a cold stress. Subsequently, the animals were sacrificed and the brains prepared for histological examination.

Major Findings: The temperature records are now being analyzed for correlations between body temperature and ambient temperature. The preliminary analysis indicates that pinealectomy delays the onset of thermoregulation in baby chicks. The initial response to cold stress in control, experimental, and sham animals is comparable; i.e., elevated body temperatures cannot be defended as ambient temperature drops. Within 2 weeks, however, control and sham animals show greater resistance to body temperature change in the cold than do pinealectomized chicks. In many trials there is a high correlation between decreasing air and body temperatures. The histological examination has confirmed that the pineal was successfully removed in 4 out of the 5 chicks.

Significance to Biomedical Research and the Program of the Institute: In recent years, an accumulation of comparative findings has demonstrated that melatonin secreted by the pineal gland plays a role in gonadal function and in thermoregulation. Heretofore, no experiments have tested the role of the pineal in the stress-response to cold. If the completed analysis of the present experiments bears out that the pineal has such a role, it will open up new vistas for experimentation relevant to thermoregulatory manifestations that also serve to give expression to emotion (e.g., blushing, piloerection) and mood. In regard to the latter, and in connection with the present experiments, one thinks particularly of the depression of nervous activity accompanying hibernation. From the clinical standpoint, melatonin has recently become of interest apropos of its possible role in depression.

Proposed Course: To be completed with the publication of the results.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00839-04 LBEB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

CNS Structures in Mammal-like Reptiles

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Carol Roth	Senior Staff Fellow	LBEB, NIMH
	Jan J. Roth	Senior Staff Fellow	LBEB, NIMH

COOPERATING UNITS (if any)

Dr. Nicholas Hotton, III, Dept. of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington, D.C.

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NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.9	0.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mammal-like reptiles (therapsids) are of great scientific interest because they represent the ancestral stock of mammals. Unfortunately, there exist but few endocranial casts for providing comparative information about the brain development in these animals. The present report includes a description of a natural endocast of a therocephalian, as well as additional details about the skull of a bauriomorph of a type not heretofore described.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The origin of the class Mammalia occurred among a group of reptiles quite unlike any living today. The mammal-like reptiles (therapsids) existed duuring the Permo-Triassic period 240 million years ago and were represented by a wide variety of both carnivorous and herbivorous forms on the super-continent Pangaea. The purpose of this project is (1) to examine the evolution of brain structure as reflected in the form of endocasts prepared from fossil therapsid skull material, and (2) to learn if any common similarities exist between gross brain morphology of therapsids, and early and recent mammals (see Project No. Z01 MH 00839-03 LBEB).

Methods Employed: The process for preparing fossil material and making endocranial casts of the brain space involves standard preparation techniques described earlier (see Project No. Z01 MH 00839-03 LBEB). Briefly, the instructions are (1) gently remove matrix from fossil skulls already separated along natural fracture lines; (2) carefully prepare intracranial surface by removing matrix and surface residue from roofing bones; (3) cast the intracranial space in any of several casting mediums suited to the fragility of the current specimen; and (4) remove cast, allow to cure, obtain measurements of volume and of such gross features as the presence and location of cerebrum; optic lobes; cerebellum; exit of cranial nerves from the endocranium; and the association between the constituent parts of the brain and the enclosing bones associated with each brain part.

It is not generally recognized by the non-preparator that the fragility of fossil material, the hardness of the matrix enclosing each fossil, and the extraordinary care with which each fossil must be handled, require a lengthy preparation time before any fossil skull is ready for analysis. Of six specimens loaned for study by Dr. Nicholas Hotton, III, Research Curator at the Smithsonian Institution (see Z01 MH 00839-03 LBEB), No. 61/53 Sesmodon and one Diictodon are now partly ready to be described in the present report. We also include the description of a natural endocast of a therocephalian.

Major Findings:

Specimen #1 (61/53). After major preparation, we have tentatively identified specimen #1 (61/53) as a small baurid, instead of a cynodont as the field records indicate. Some of the visible diagnostic features which separate this specimen from other theriodonts are (1) the superior orbital border is composed of prefrontal, frontal, and postorbital bones which is an association not found in cynodonts; (2) contrary to gorgonopsians, the preparietal is absent; (3) the postcanine teeth extend along the length of the maxilla and are not abbreviated as in gorgonopsians; (4) the postcanine teeth are not gomphodont (peg-like) as in baurids; however, the occlusal surfaces are not yet prepared, preventing a definitive determination of their condition; (5) the postorbital bar is fully developed, slender, and makes contact with the squamosal below the temporal opening, rather than

above as in advanced theriodonts; (6) the lacrimal is large, which is uncharacteristic of therocephalians; (7) unlike therocephalians, the postfrontal is lacking; (8) the canine on each side is single and not double as in many primitive therocephalians; (9) the pineal foramen is present and large (both conditions are rare among baurids, but do occur); and (10) the phalangeal formula appears to be 2, 3, 3, 3, 3; however, the manus is damaged and not clearly represented.

Since the palate is still unprepared, it is not possible to ascertain the presence of pterygoid vacuities which would place this specimen among the baurids. Baurids are derived from therocephalian antecedents, and the shared suite of characters listed above indicate that specimen #1 (61/53) is either a very advanced therocephalian or a primitive baurid. Granted the absence of comparative palatal or postdentary information, the other findings (pineal opening, the absence of a postfrontal, the single canine, the large lacrimal, and the configuration of the orbital bones) strongly suggest a new species of baurid theriodont. The endocranium is very fragile, spalls badly, and as a consequence, is not yet prepared.

Although bauriamorphs were at one time thought to have given rise to believed the Tritylodontidae, the latter are now believed to be very advanced cynodonts. Accordingly, the baurids appear not to be directly related to mammalian origins. The specimen described above will, with further study, most likely be described as a new species of baurid, after which, according to the Smithsonian's agreement with South Africa, it will be returned to its country of origin.

Natural endocast of a therocephalian. Endocasts of therocephalians, which are the antecedents of bauriamorphs and primitively related to cynodonts, do not exist. We have recently been fortunate to discover with Dr. Nicholas Hotton an unprepared natural endocast of a therocephalian in the collections of the Smithsonian Institution. A natural endocast is one in which the roofing bones have disappeared, revealing an endocast of the brain as preserved by the original matrix. This endocast, like those of cynodonts such as Thrinaxodon, illustrates that the theriodont lineage leading to mammals had developed large olfactory bulbs which had a short peduncle connecting them to the cerebrum which was smaller and much narrower. The floccular area of the cerebellum is large in comparison with other reptiles. Auditory acuity must also have been good, as is indicated by the relatively small size of the postdentary bones that become the middle ear bones of mammals. The pineal body and third eye are conspicuous in primitive theriodonts, although the third eye disappears at about the same time as the mammalian transition from therapsids. We have postulated (Project No. Z01 MH 00839-03 LBEB) that this event is related to the acquisition of endothermy.

Diictodon #191. In contrast to the theriodonts, our preparation of endocasts from the largely herbivorous anomodont group, and particularly Diictodon, illustrates that the olfactory bulbs of these animals were not

nearly as well developed as are those of cynodonts, nor is the cerebral area as large. The pineal eye was prominent and much larger in most anomodonts than in theriodonts.

Significance to Biomedical Research and the Program of the Institute: We conclude from existing endocasts that advanced cynodonts had a good sense of smell (olfactory bulbs), an advanced degree of motor ability (flocculus), and as others have demonstrated, well-developed hearing. As we have suggested elsewhere, some theriodonts may have been endothermic. We suggest that advanced cynodonts with well-developed olfactory acuity, hearing, and agility, may have lived nocturnally. It may well be that the earliest mammals were not forced into a nocturnal existence by dinosaurs as is commonly believed, but rather that these animals were already using nighttime niches well before the advent of dinosaurs.

Finally, although there appears to be somewhat more encephalization among the ancestors of mammals (the cynodonts) than among such cynodont contemporaries as the herbivorous dicynodonts, the brains of both groups are small and lie well within the reptilian level of encephalization. The endocast of Bienotherium, an Upper Triassic tritylodont described by others, does not demonstrate any marked enlargement of the cerebral areas. Consequently, the expansion of the neocortex characteristic of modern mammals probably did not occur until well after the demise of ancestral therapsids in the late Triassic.

In summary, and in line with our earlier findings, and those previously reported by others on other forms of therapsids, examination of the present brain casts indicates that the cerebrum of cynodonts and dicynodonts was small, long, and narrow, and that the olfactory bulbs were larger than any other brain region. The parietal foramen in dicynodonts is considerably larger than in cynodonts; housed a well-developed third eye within the cone-shaped foramen; and is indicative of an equally well-developed epiphyseal system in the epithalamic region of therapsids. Encephalization of all therapsid groups appears upon examination to lie well within the reptilian range and does not demonstrate the enlargement of the forebrain characteristic of mammals.

Proposed Course: To be completed upon publication of the results in the book emanating from the LBEB Conference on Mammal-like Reptiles.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00842-04 LBEB

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Comparative Neuroanatomy of the Parietal-Pineal Complex in Lizards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Carol Roth	Senior Staff Fellow	LBEB NIMH
	Jan J. Roth	Senior Staff Fellow	LBEB NIMH

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1.2	0.9	0.3

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project deals with the anatomy of lizard epiphyseal systems and the relationship of the central nervous system to the endocranium in modern reptiles. Anatomical and morphological bases for functional differences in lizards that display varying degrees of development of the epiphyseal complex are explored. Parietal foramen closure with increasing size (age) in the lizard Anolis carolinensis cannot be compared directly to parietal foramen closure in the stem-reptile of 300 mya, Captorhinus aguiti. The ratio of brain volume to endocranial space volume is currently being quantified in 17 size ranges of the lizard Anolis carolinensis and in Alligator mississippiensis. These data will provide a first analysis of such relationships using histological techniques. The forthcoming information is expected to extend an understanding of encephalization quotients and their application to brain evolution.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The mammalian pineal body is part of a complex of epithalamic structures, including a third eye, that find their most extensive development in lizards and fossil vertebrates such as the therapsid (mammal-like) reptiles. The gross and microscopic anatomy of reptilian pineal systems can be used to derive models for the evolution of mammalian pineal systems. The present project has two objectives:

1. Anatomy. The development of the epiphyseal system is being examined in embryos of parietal-eyed and parietal-eyeless species of lizards to evaluate the comparative ontogeny of epiphyseal development.

2. Morphology.

(A) The endocranial space and its relationship to brain growth is being compared through the use of endocasts and histological material in known size (age) stages of the lizard Anolis carolinensis and the alligator, Alligator mississippiensis, to determine comparative encephalization rates.

(B) Because age related changes in parietal foramen closure occur in the lizard A. carolinensis, foramen closure will also be examined in fossil reptiles as a means of determining age.

Methods Employed:

1. Anatomy. The brains of Cnemidophorus velox, C. tigris, Sceloporus undulatus, and Uta stansburiana are prepared with routine paraffin methods to compare intraindividual variation in gross pineal morphology. Paraffin embedded tissues are stained (Bodian or Kluver and Barerra) to visualize neural elements and cranial structures.

2. Morphology.

(A) The brain to brain space ratio (brain volume/endocranial space volume X 100), usually considered to be 50%, is being compared in lizards and alligators as they mature. These data are acquired in two ways: (1) measurements (weight and volume) of fresh brain and silicon rubber endocranial casts are compared; and (2) planimetric measurements of histological sections of the heads of Anolis carolinensis cut in the horizontal plane are used to compare and measure volume (cm^3) of the whole brain, its parts, and the brain space. With paraffin embedding, there is a shrinkage of nervous and other tissue. Measurements are to be obtained for volumetric comparisons of fresh tissue and histologically prepared material. The data are expected to afford a modification of the Jerrison formula, $EQ_i = E_i/E_e$, which assumes 50% of the endocranial space is occupied by brain tissue and where EQ_i = encephalization quotient, E_i = actual brain size, and E_e = expected brain size.

(B) Parietal foramen closure is examined in 107 prepared skulls of the lizard A. carolinensis, in histological sections of 90 individuals of the same species, and in 6 prepared skulls of the stem reptile of 300 million years ago, Captorhinus aguti, loaned to us from Dr. John Bolt, Chicago Field Museum. Major skull table elements (frontal, parietal, and nasal bone lengths and widths) are used to examine the influence of any allometric relationship between parietal foramen size (closure) and growth of the skull.

Major Findings:

1. Anatomy. The survey of the epiphyseal system of the lizards A. carolinensis, S. undulatus and U. stansburiana indicates that the parietal eye is in contact with the pineal body via a rostral extension of the pineal body that courses to the parietal eye dorsal to the cerebral hemispheres in these parietal-eyed species. Since the parietal eye and pineal body arise from the same embryological anlagen in all living parietal-eyed forms that have been reported in the literature, and if one may assume that this interpretation of anatomy holds for fossil forms that have parietal foramina, then it appears that this relationship is also true of epiphyseal systems in fossil vertebrates where the intracranial skull roof is preserved well enough to permit close examination of the structure of the parietal eye tube and cranial roof of the parietal bones. As reported earlier (Project No. Z01 MH 00842-03 LBEB) the pineal bodies of unhatched lizard embryos (C. velox) which lack one or both lateral eyes, appear to be smaller in size than the pineal body of a normal embryo of the same development. The pineal body of an abnormal embryo has a saccular appearance, while a pineal body of a normal embryo looks very similar to the compact secretory pineal characteristic of adults. It is possible that a trophic relationship between the visual system and the pineal gland may exist, but this area is as yet, unexplored.

2. Morphology.

(A) Our previous results (Project No. Z01 MH 00842-03) indicated that the brain fills approximately 85% or more of the endocranial space in young alligators (4 animals examined to date) and an estimated 70% in young lizards (A. carolinensis). Although the study is not complete, we can now extend these observations to one size range of the brains of sexually mature (46mm snout-vent), female A. carolinensis, which occupy approximately 54% of the endocranial space. Based on planimetric measurements on 5 animals, the cerebrum occupies a greater amount of the endocranial space (27.4%) than the remaining structures (26.6%). These percentage values, although preliminary and restricted to one size range of adult females, indicate that the cerebrum and its representation on the endocast may more accurately reflect encephalization during the evolution of the brain than does the whole brain encephalization quotients currently being used.

There remains the difficulty of determining the extent of cerebral representation on the endocasts of fossil vertebrates. The relatively greater discrepancy found between brain and brain space in younger and older individuals of existing reptilian species may prove to be important. When a single fossil animal of unknown age is used to establish encephalization quotients for a whole species or family of fossil vertebrates without considering the animal's age and without data indicating that a discrepancy between brain and brain space may exist with age, misleading quotients may be the result. The new values being obtained should provide a more realistic basis for estimating encephalization quotients in living and fossil vertebrates, which in turn will prove helpful in determining the levels of encephalization in some mammal-like reptiles which are significant because of their close proximity to the evolutionary origin of mammals.

(B) Our description of parietal foramen closure in A. carolinensis and Captorhinus aguti (Project No. Z01 MH 00842-03 LBEB) will be published in the forthcoming book resulting from the Conference on Mammal-like Reptiles organized by this Laboratory. Briefly, parietal foramen closure in small lizards appears to follow a predictable sequence as described earlier with increasing lizard size and presumably age. Closure of the foramen may also be correlated with reproductive variability as age increases, but a causative relationship remains to be explored. Parietal foramen reduction in size also appears in the stem reptile, C. aguti, but the pattern does not follow the pattern observed in the lizard A. carolinensis. Parietal foramen reduction appears to be an occurrence in fossil vertebrates (e.g. Daptocephalus, see Project No. Z01 MH 00842-03 LBEB) with a third eye, as it is among living reptiles; however, the lack of adequate growth stages among individuals of fossil species will leave this question unanswered until additional material becomes available.

Significance to Biomedical Research and the Program of the Institute: This project provides an evolutional approach to a better understanding of the comparative origins of pineal systems in reptiles and in mammals. A functional or trophic relationship between the ontogeny of the visual system and the photoreceptive pineal gland is suggested by the abnormal pineal body observed in hatchling lizards that developmentally lack lateral eyes.

The study of encephalization quotients and sensory system involvement in species evolution can be aided by the use of endocranial casts and brain-to-brain space ratios calculated from histologicaly sections of certain lizards and the alligator. Such casts and sections demonstrate a negative allometric relationship between the brain and brain space that increases with increasing size and presumably age of the individual.

Patterns of ossification and closure of the parietal foramen in parietal-eyed lizards and fossil reptiles may prove to be correlated with size and age. This information could provide a means for determining age and growth stages in reptiles, both living and fossil.

Proposed Course: To be completed with publication of the results, part of which will appear in the book to be published from the Conference on Mammal-like Reptiles organized by this Laboratory.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00846-03 LBEB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effects of Sex Hormones on Iron Content of the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Joanna M. Hill Biologist LBEB NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

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NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.7	0.3

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Sex hormones affect many aspects of iron metabolism, including the uptake, storage, and mobilization of iron. In the present experiments on rats it has been further demonstrated by spectrophotometry that during proestrus the concentration of iron in the globus pallidus and substantia nigra about doubles relative to the other three stages of the estrous cycle. Iron levels also peak during the first third of pregnancy when the concentration of progesterone is high.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The present study relates to the main interest of this Laboratory in the functions of the striatal complex (see also objectives of accompanying report, Z01 MH 871-04 LBEB). Preceding experiments on rats suggested that iron levels of the brain vary with the natural fluctuations of ovarian hormones during the estrous cycle and during pregnancy. In the present work further observations are being made with spectrophotometry in order to learn whether or not the concentration of iron in the pallidal part of the striatal complex and in the substantia nigra varies during the estrous cycle and during pregnancy, and what effect, if any, gonadectomy and estrogen replacement have on brain levels of iron.

Methods Employed: Samples of globus pallidus (GP) and substantia nigra (SN) obtained by micropunch from 270 μ thick sections of rat brain are pooled so as to form a sufficient sample for spectrophotometry. These samples are compared with those from the "iron-poor "cortex. Iron levels in the serum and liver are also determined for each animal. The following groups of rats have been examined: (1) females in each of four stages of estrus, (2) females sampled every 4 days throughout pregnancy, as well as 15-24 hours postpartum, (3) ovariectomized females, (4) ovariectomized females with estrogen implants, (5) intact males, (6) castrated males, and (7) castrated males with estrogen implants.

Major Findings: During proestrus (a time of peak concentration of ovarian hormones) the iron concentration of the globus pallidus and substantia nigra approximately doubles relative to the levels during the other three stages of the estrous cycle. Brain iron levels also peak during the first third of pregnancy, a time when progesterone concentrations are high. Although brain iron diminishes in the latter part of pregnancy, it does not undergo the depletion that occurs in liver iron. Ovariectomy at 4 weeks of age, and estrogen treatment for 3 weeks before sacrifice (12th week), has little effect on the females' brain iron. Gonadectomy in male rats, however, results in an increase in brain iron. Apparently testicular hormones suppress iron accumulation in the globus pallidus and substantia nigra.

Significance to Biomedical Research and the Program of the Institute: This year's findings provide additional evidence that brain iron fluctuatations correlate with changes in endogenous sex hormones, suggesting a role for iron in neuroendocrine functions. Reference should be made to the section on "significance" in an accompanying report (#871-05) regarding the histochemical location of iron in the brain. As there noted, the distribution pattern of iron in the hypothalamus suggests that it is involved in some way with the metabolism of neuroactive peptides and with the neuroendocrine regulation of LHRH. Apropos of the present experiments, it may be relevant to point out that the increase of pallidal iron during proestrus occurs in a region not far from the medial preoptic area, a site where iron salts or electrical stimulation with steel electrodes results in ovulation.

The results of the present project suggest a relevance to factors affecting the physical well-being and mental health of human beings. As is well known, iron deficiency is a prevalent nutritional disorder resulting in many diverse manifestations that include anemia, stunted growth, weakness, anorexia, amenorrhea, menstrual irregularity, irritibility, apathy, listlessness, inattention, and decrease in scholastic performance. In laboratory animals iron deficiency results in a reduction in total activity, a reversal in diurnal activity, and a deficiency in learning performance. It is possible that some of the described behavioral changes are owing to insufficient iron for iron-requiring neurotransmitter systems as, for example, monaminergic systems. Moreover, the fluctuations of iron in the above-mentioned critical brain areas during the estrous cycle might be conducive in iron-deficient individuals to amenorrhea and menstrual irregularity. There are equally challenging questions in regard iron fluctuations during pregnancy.

Proposed Course: To be continued.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00871-05 LBEB

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

A Histochemical Study on the Location of Brain Iron

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Joanna M. Hill Biologist LBEB NIMH

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The application of a modified Perl's stain in conjunction with a diaminobenzidine method has revealed fine details regarding the location of brain iron that were heretofore unknown. The iron is seen to occur in granules, amorphorous accumulations, and fiber-like processes in glial cells and neurons. There appears to be an interesting correlation between (1) the high concentration of iron in structures known to have a high content of GABA (globus pallidus and substantia nigra) and (2) the distribution of iron in sites in which neuroactive peptides have been localized, including the tuberal region of the hypothalamus.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project was originally undertaken in connection with ongoing investigations of the Laboratory on the functions of the striatal complex, a fundamental part of the forebrain in reptiles, birds, and mammals. The pallidal part of the striatal complex contains a conspicuously greater amount of iron than any other structure in the forebrain. The present project deals with the findings afforded by a modification of a recently described stain that combines Perl's histochemical technique for iron with a diaminobenzidine method (Histochem., 66: 239-244, 1980). The resulting histological picture provides much more detail not only about the location of iron in the globus pallidus, but also in structures in which iron concentration is low.

Methods Employed: The intensification procedure is based on the catalytic nature of ferric ferrocyanide, formed from endogenous iron and the acidic ferrocyanide of the Perl's reaction. Ferric ferrocyanide catalyzes the polymerization and oxidation of benzidines by peroxide. Following Perl's reaction (immersion of tissue in a 1:1 solution of 2% HCL and 2% potassium ferrocyanide for 10 minutes), the tissue is washed in water for 5 minutes, followed by immersion in a 0.05% solution of diaminobenzidine (DAB) in 0.1 M phosphate buffer pH 7.4. After 20 minutes of incubation, a 1% solution of hydrogen peroxide is added to the DAB to make a 0.1% solution. The tissue is incubated for another 20 minutes and then washed with distilled water. The brown reaction product lends itself both to light and dark-field microscopy. The brain sections are counterstained with thionine.

Major Findings: With the present modification, the areas already known to be richest in iron (i.e., the globus pallidus and substantia nigra) stand out in bold contrast. Indeed, in the rat the reaction is so intense that it is easy to distinguish the brain of a female (with its higher content of iron) from that of a male. Other deeply staining structures include the paleopallidum and islands of Calleja (of the olfactostriatum), the interpeduncular nucleus, and the cerebellar nuclei.

Upon microscopic examination, the iron is seen to occur in granules, amorphous accumulations, and angular fiber-like processes in glial cells and neurons. For example, fibril-like structures can be seen in the radiate fibers of the globus pallidus.

Some of the areas with a lower content of iron (found also in glia and fibers) include the ventral thalamus, inferior and superior colliculi, central amygdala, and lateral habenula. In certain structures iron occurs in bouton-like elements on or in the perikarya of neurons. These structures include: the lateral septum, the bed nucleus of the stria terminalis, and (less predictably) ventral pallidum, diagonal band, an area lateral to the anterior hippocampus, and neocortex. Iron appears as a fine granular dusting in the cells of the supraoptic, paraventricular and suprachiasmatic nuclei, and occasionally in the cingulate cortex and dentate gyrus.

In the circumventricular areas lacking the blood brain barrier, iron occurs in high concentration, being present in granules, amorphous accumulations, and fiber-like processes (subfornical organ, organum vasculosum of the lamina terminalis, median eminence, subcommissural organ, pineal, and pituitary). The outlines of the tanycytes of the third ventricle appear particularly striking.

Significance to Biomedical Research and the Program of the Institute: Prior to summarizing histochemical findings of special interest, it should be noted that most brain iron occurs in as yet unidentified forms. A portion occurs in the iron-storage form, ferritin, and a smaller fraction in the form of enzymes. The iron-containing enzymes aconitase and succinic dehydrogenase are involved in the metabolism of glutamine, GABA, glutathione and glutamate. Iron is required for the rate-limiting enzymes tyrosine hydroxylase and tryptophan hydroxylase, monoamine-synthesizing enzymes. Aldehyde oxidase and monoamine oxidase (monoamine degrading enzymes) require iron for normal activity. Iron may also be involved in the storage, transport and receptor mechanisms of the monoamines. Given these considerations, the following points are of special interest:

1. GABA is highly concentrated in those areas where iron is localized in glial cells and fibers, being particularly notable in the globus pallidus and substantia nigra.

2. The pattern of distribution of iron, however, correlates to a greater extent with the distribution of neuroactive peptides, and in some areas iron and a peptide appear to be within the same distinct structures. Both iron and enkephalin are in the highest concentration in the globus pallidus, and both are present within fibers, including those destined for the olfactory tubercle.

3. The distribution pattern of iron and enkephalin in the hippocampus and dentate gyrus are identical.

4. The distribution and size of iron-containing granules in the ventromedial hypothalamus and median eminence are similar to that of the peptide-containing neurosecretory granules as stained with the classical neurosecretory stains. Although iron and many peptides are found in the periventricular areas of the hypothalamus and median eminence, it is only in the rodent brain that LHRH has been identified in the tanycytes--structures shown here to contain iron. Hence, the distribution pattern of iron suggests that this metal is involved in some way with the metabolism of neuroactive peptides and with neuroendocrine regulation.

5. Enkephalinase (a metalloenzyme inhibited by iron chelators) has a distribution pattern similar to enkephalin, opioid receptors, and iron. Perhaps the metal in enkephalinase is iron, linking iron to the metabolism of yet another neuroactive compound.

Proposed Course: To be continued.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00834-05 LBEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Group Organization in Rodents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John B. Calhoun	Research Psychologist	URBS LBEB NIMH
Other:	James L. Hill	Visiting Associate	URBS LBEB NIMH
	Garrett A. Bagley	Computer Programmer	URBS LBEB NIMH
	Charles L. Bishop	Biologist	URBS LBEB NIMH
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COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two populations of rats (Rattus norvegicus) were allowed to increase to three times optimum density. Painting one side of the habitat a darker color sufficed to divide the population into two subgroups, of which rats living on the dark painted side became dominant. Rats spent 90% of their time on their respective sides, thus enhancing (intra) group stability, and reducing (inter) group conflict. A 7-generation, 5-year study of a mouse (Mus musculus) population was also completed. When the population reached 8 times optimum density in the 6th generation (G6), 75% of G7 weaned came from 4 contiguous cells of the circular 8-cell habitat. Behavioral sink crowding together of mice in the other 4 cells continued through the next 8-month breeding period, with accentuated aggregation of mice in the nest boxes of all cells sufficient to preclude survival of any G8.

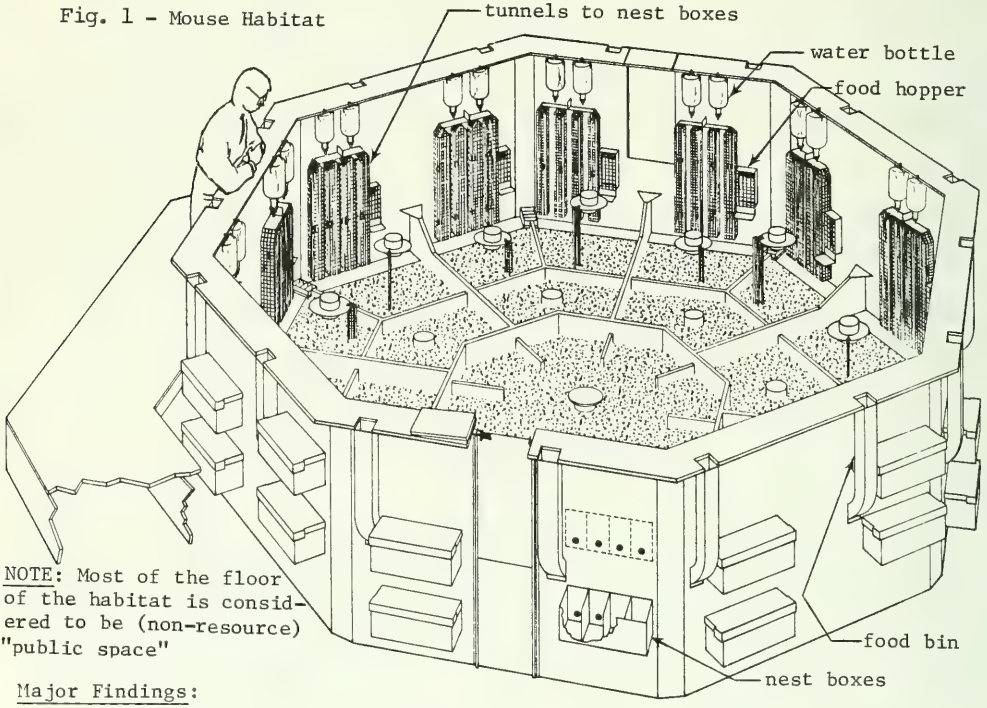
PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine how maintenance of spatially localized subgroups contribute to population stability in the face of long-term high density situations.

Methods: Mice (Mus musculus) and rats (Rattus norvegicus) of high diversity genetic background were maintained in large complex habitats (see Fig. 1 for mice, and see Z01 MH 00836-05 for rats). Focus here was placed on the 6th and 7th generation of mice which lived in a radially symmetrical 8-cell habitat, over a year during which the population declined from 8 to 2 times optimum density. The rat population lived in a bilaterally symmetrical habitat with anterior, resource-availability induced, dominance for 18 months. During this time the population declined from 3 to 2 times optimum density. Periodic surveys of capture location for mice, and automated recording of locations for rats, provided the data considered here.

Fig. 1 - Mouse Habitat



NOTE: Most of the floor of the habitat is considered to be (non-resource) "public space"

Major Findings:

I. Study 133 (Mice): Emphasis here is placed on conditions influencing inability of any mice to rear young of the 8th generation during the life span of the 7th and terminal generation (See Project No. Z01 MH 00835 -05 LBEB for results on reproduction). 7th generation (G7) mice were born under conditions of severe crowding and only enough survived to maintain adults at 8 times optimum density when G7 first bred. During the

entire life span of G7, optimum association among adults occurred only within the nest boxes (2% of the space earlier occupied by an optimum sized group), where on the average each mouse associated with 8-11 others. Even this extreme spatial withdrawal (an attempt to preserve the minimum level of optimum associations) was at the expense of the ejection of 70% of the males to live in large aggregates in public space, and for them to become characterized by extreme withdrawal and fragmentation of behavior. All this behavioral pathology was aggravated by accentuation of development of a behavioral sink (movement of mice to sites increasing contact with others) in 1/2 of the habitat as shown in Fig. 2. This curve presents the interaction of concentration of mice in cells in the lower box levels of cells, and the increase in males accepted into nest boxes. Spatial variability in production of weaned G7 (Fig. 2) provides support to the conclusion that accentuation of the behavioral sink further inhibits production and survival of pups.

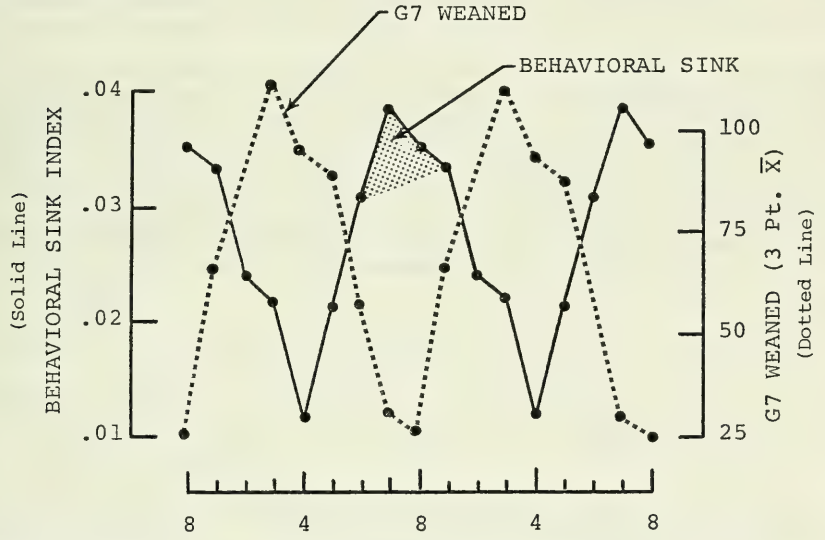


Fig. 2

II. Study 140 (Rats): Throughout the study every rat exhibited marked consistency of residence on either the light-painted or the dark-painted side of the habitats. Residents of the dark side were uniformly dominant over those on the light side. On the average, 90% of a rat's time was spent on the side of normal residency. The dark side group averaged 14 rats, (5.5 males, 8.5 females) in contrast to 19 for the light side (11.5 males, 7.5 females). Thus this single difference, degree of light reflection, facilitated segmentation of the population into two subgroups, which exceeded optimum density by 17% and 58%, repectively. Our extraordinary recording system, which continuously monitors the location of all members of the population by their identity, provides a degree of quantification

and accuracy that is unusual for studies such as this.

Significance to Biomedical Research and the Program of the Institute:

As demonstrated in our previous studies, rodents share with humans the need to be members of groups of about 12 individuals. Under severe crowding, such small groups of humans might also retreat to some minimum space to preserve the rewards of intimate interaction with others, even though restriction of most activities to such a small portion of optimum behavioral space may have deleterious consequences. If so, this pathological process has particular significance for institutions, such as prisons and places for housing the elderly or mentally ill, where society decrees that groups may be confined to small spaces lacking many of the prerequisites for optimal behavioral expression. Our studies with rats indicate that seemingly insignificant environmental changes, such as differences in light absorption of walls, etc., can assist in ameliorating effects of crowding by helping to define a smaller subset of others with which any individual interacts.

Future Course: Structuring the computer data tapes for these two 5-year studies will be completed by December 1981. The next three years will be devoted to the analysis and integration of these very large data bases, which include information related to several important problems besides the ones emphasized above, among which are: (1) experiential histories of subjects influencing settlement in either the behavioral sink half of the habitat or the half where normal behavior and reproduction persisted longest; (2) size, constancy and composition of subgroups inhabiting cells and subcells, and extent of range of members during times of activity.

Publications: None.

Honors: (to principal investigator)

1. Received an ADAMHA Administrator's Award for Distinguished Service.
2. In the fall of 1981 is scheduled to review his past 20 years of research on the CBS-TV, Walter Cronkite science commentary program, Universe.
3. To present six invited lectures, November 6-13, 1981, before the American Academy of Pediatrics.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00835-05 LBEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Crowding and Population Dynamics in Rodents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	John B. Calhoun	Research Psychologist	URBS	LBEB	NIMH
	Garrett A. Bagley	Computer Programmer	URBS	LBEB	NIMH
	Charles L. Bishop	Biologist	URBS	LBEB	NIMH
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COOPERATING UNITS (if any)
None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.4	1.1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary goal of the project is to determine the behavioral, ecological and reproductive factors which characterize a population of house mice (Mus musculus) at each of several successive stages of crowding. The rate of population growth was regulated; after the adult population reached 100, the population was allowed to double approximately every 200 days. After 1200 days the population reached about 1600 and then began to decline because the death rate exceeded the rate of recruitment. The rate of reproduction began to further decline with no survival of pups beyond three days of age, and the population size decreased to about 1200. Removal of all but the youngest generation of animals, which reduced population size to less than 500, a size at which a high rate of reproduction had occurred earlier, did not stimulate reproduction and the population continued to decrease until the study was terminated. The tendency to remain in highly crowded groups even after the population size was reduced was the primary cause of the collapse of reproduction because the mice exhibited poor and abnormal parental behavior under highly crowded conditions.

Project Description:

Objective: To determine if a population of mice subject to slowly increasing crowding over several overlapping generations would reach a stage where a reduced reproductive recruitment of new members would suffice to threaten population extinction.

Methods: Prior methods of controlled staging of population recruitment through reproduction continued with an attempt to double the size of the total population each 200 days from progeny born within a restricted 60-day period. By this strategy, each successive generation is produced by experienced prime reproductive aged survivors of the two prior generations. At the time of inception of births that could be called the 7th generation (G7) there were 1600 mice in the adult population (8 times optimum density). Because of poor survival of young, the duration of time allowed for recruitment of G7 was extended from 60 to 187 days.

For the same reason the initial attempt to form an 8th generation (G8) was extended to 140 days. By the end of this time the older adult population had declined from 1128 to 790 due to death with no replacement by reproductive recruitment. Therefore the older adults were removed, leaving only members of the youngest, and most reproductively active 7th generation. At the end of another 147 days of unsuccessful reproduction the study was terminated.

In addition to the regular surveys over the last 287 days of reproductive history involving G7 (during which data included status of pregnancy, lactation and litters) intervening surveys were conducted to determine the status of litters.

Sixteen pairs of G7 were removed from their crowded setting at 50 and 250 days of age and placed as pairs in special optimally structured breeding cages where they were left for a sufficient time to rear two litters. In addition, the only two G8, both females, that survived to weaning were removed and placed with G8 males, born earlier to G7 in the special breeding cages.

Major Findings: At the beginning of the formation of a 7th generation, the adult population contained 1600 individuals, 8 times optimum density. This time marked the beginning of reproductive failure of the population. Only 556 G7 (born over a 187-day span of time) lived to sexual maturity. Nine times as many progeny per female lived to sexual maturity during the formation of all prior generations.

No progeny born to G5 to G7 females survived to weaning during the first 140 days of attempt to form an 8th generation. By the end of this time the number of older G5 and G6 had declined from 1600 to 800. An increased female mortality, expressed by deaths just before parturition, caused the ♂:♀ sex ratio to change from 1.0:0.90 to 1.0:0.69 during the above 140-day breeding period.

During the last 147 days of the study when there were only G7 present,

only 2 pups out of the many litters born survived to weaning. These were the only survivors out of 1531 litters estimated to have been delivered over the 287-day span of these two breeding periods.

Very few pups survived beyond three days of age. Since very little milk was observed in the stomachs before death, failure to be nursed must have contributed to death. Abnormal adult behavior, such as cannibalism, desertion and trampling, all of which are accentuated by high density crowding, no doubt contributed to this infant death, and thus fostered population extinction.

Nearly all pairs of mice of G3 through G7, removed from the habitat at 50 days of age and placed as pairs in small habitats, reared two litters successfully. Most pairs of G7, removed at the end of the first breeding period also successfully reared two litters. It therefore appears that the failure to produce an 8th generation in the larger habitat is related more to conditions of sustained crowding, rather than permanent impairment of behavior.

Significance to Biomedical Research and the Program of the Institute: This report concludes the data gathering phase of the second of two large scale, long term, studies with mice. Comparative results have important implications for both further experimental population research with mammals, as well as for the human scene. When maturation occurs within a setting of 6-8 times optimal density, reproductive recruitment is insufficient to prevent extinction. This insufficiency is preceded by development of a wide range of behavioral pathologies that result in reduced expression of individual potential as well as disruption of intra- and intergroup organization.

Where population increase (as in the earlier study) was uninhibited by predation, disease, or investigator prolongation of inter-generation time, the available time for incorporating the increasing number of experiences finally proved so inadequate that members of the last generation so lost ability to develop any effective relations with associates, including mating, that all were essentially autistic. However, when time and opportunity for incorporation of experience was increased (as in the currently completed study) by (a) environmental design that protects intra-group integrity, and (b) investigator prolonged inter-generation time, the final impact of sustained overcrowding fell more on a final generation where disorganization of earlier more integrated behavior totally destroyed care and protection of young by parents and associates. This latter pathology became accentuated by the "behavioral sink" pathology of the acquired need for close association with others which culminated in extremely dense clusters in some places with no animals present in other similar spaces.

Future course: Full structuring of the computer data files will be completed by December 1981. Following that, integrative analyses will be made with comparisons to the earlier study.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00836-05 LBEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Conceptual Adaptation and the Amelioration of Crowding

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	Garrett A. Bagley	Computer Programmer	URBS LBEB NIMH
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COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.0	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

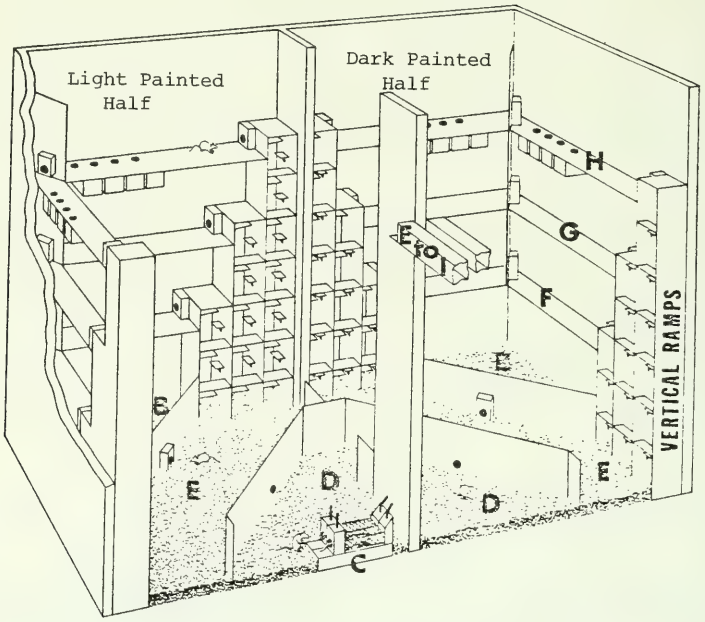
Rats (Rattus norvegicus) living in complex habitats with designed communication networks develop capacities for "hypothesis building" that facilitate more effective interactions with associates. Required acquisition of cooperative behavior reduces stress, even at above optimal population densities. Rats also exhibit the capacity to acquire non-experimenter-required cooperative and altruistic behaviors, as well as to physically modify access to routes of communications between segments of the environment inhabited by different subgroups. Such "hypothesis-building" activities have dual consequences of fostering longer duration, more complex, behaviors, and buffering the deleterious consequence of above optimal densities.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine if the increase in conceptual capacity derived from life-span demand for engaging in cooperative behaviors ameliorates stress related pathologies associated with living at high densities.

Methods utilized: Two populations of rats (Rattus norvegicus) were each housed in separate identical complex habitats (schematically shown below) for 18 months. Members of the experimental population were required to learn difficult cooperative behaviors in order to obtain food and water. Eighteen surveys of the 18 females in each group provided data on health and reproductive performance. Automatic recording of duration of behaviors provided the data to evaluate the hypothesis that acquisition of cooperative behaviors increase conceptual capacity, at least to the point of permitting longer sequences of the acts which comprise a behavioral state.



Major Findings: Two sets of findings are particularly instructive.

Reproduction. (a) The incidence of pregnancy and the survivorship curves for pups beyond the first day of birth was essentially identical for both experimentals (EXP) and controls (CON). These data suggest equivalent competence of EXP and CON with respect to reproductive and maternal behavior. (b) 2.4 times as many pups were found in the CON habitat, and on these days 2.4 times as many CON females as EXP females were lactating. Consideration of (a) and (b) suggest that 58% of EXP litters disappeared during the first few hours after birth [i.e.: (2.4 - 1)/2.4 = .58]. Such greater pup production by CON has characterized

the total history of this study; it is just the opposite from the original hypothesis that reproduction would be less in the controls because of anticipated greater crowding related stress that the CON would experience. Resolution of this unexpected finding may prove the key to the broad objective of understanding conceptual adaptation in the rat. A set of interacting hypotheses will further be explored following final structuring of all related data bases. We suspect that experience in moving through a complex physical space generalized to enhance adaptation to complex inter-individual interactions and thus lessened stress from them for CONTROLS. On the other hand, we suspect that the time demands on EXPERIMENTALS to maintain cooperative acquisition of food and water disrupted very early maternal care, including exclusion of access by males to newborn young. However, the fact that EXP pups maintain a 20% weight advantage over CON during the nursing period attests to EXP being better mothers of litters which do survive.

(2) Behavioral States: Durations of being in the food and water compartments were examined for a 72-hr period after maximal acquisition of cooperative behavior by the EXP. It was anticipated from earlier studies in much more confined situations that males would exhibit longer durations than females and that EXP (i.e., cooperating rats) would have longer durations than the CON. In general just the opposite occurred. The general theory holds that any conditions fostering "hypothesis building" (complex problem solving) will increase the durations of behaviors by permitting longer sequences of acts comprising a behavioral state. It is to be noted that both CON and EXP lived in very complex compartmentalized environments, where routes to a goal were often blocked by associates. On such occasions the blocked rat often immediately took an alternate route to the goal. This evidence of adaptive learning suggests that these rats may have acquired the generalization that any problem may have alternative solutions. However, the clue to these unexpected findings is provided by the CON females. These CON females spontaneously acquired two complex behaviors: (1) cooperative-altruistic action in obtaining more sunflower seed reward from a special dispensing device; (2) elaborate plugging of access channels between the two halves of the habitat. This ameliorated the contact and conflict between members of the dominant and subordinant halves of the CON population.

Future course: Much of the next 3 years (after completion of computer accessibility to all data of this study by December 1981) will be devoted to the analysis and integration of the many facets of this study. Only through such integration may anomalies, such as reported above, be resolved.

Significance to Biomedical Research and the Program of the Institute: These studies with rats demonstrate the character of processes which likely were operative in the progressive development of human culture and the enhancement of adaptive capacity and promotion of human welfare. The generic capacity for hypothesis building is enhanced by movement through a network route environment having bilateral symmetry and anterior dominance of demands on behavior. Furthermore, opportunities for acquiring cooperative and/or altruistic behavior, or of modifying routes of contacts among subgroups has the dual effect of reducing conflict and enabling acquisition of more complex and longer duration behaviors. Derived principles from these studies should be applicable to the human scene.

Publications: None. (At the time of the design of this study in 1975, it was realized that attention to analysis could not begin until its completion in 1981 and after the structuring of the data base.)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00837-05 LBEB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Vocal Communication in Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

Research Services Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.7	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Vocal communication among members of free ranging populations of rats housed in complex laboratory enclosures are either audible to humans, or are above 20 kHz, the upper limit of human hearing (ultrasonic). Audible vocalizations are frequently uttered as one, or a short series of squeals by a rat when attacked or confronted by another. Ultrasonic vocalizations are uttered during attacks and confrontations during naso-naso, naso-anal, and naso-genital inspections, and as part of male post-copulatory behavior. When two, or more, rats are vocalizing simultaneously they are capable of utilizing separate frequency bands so that each signal is distinguishable. Vocalizations of a population forced to perform cooperative behaviors to obtain food and water are being compared to those of a population for which food and water are freely available. An extensive library of vocalizations is being prepared for correlation with direct observations of behavior and computer records of movement patterns to determine if those rats required to perform cooperatively exhibit different and, possibly, more complex patterns of vocalization and behavior than those rats with free access to food and water.

Project Description:

Objectives: To determine if the quality, quantity and pattern of rat vocalizations change when the animals are required to perform complex cooperative behaviors to meet their physiological needs.

Methods: Vocalizations are detected by a B&K condenser microphone (Dynamic range = 20 kHz) equipped with a parabolic reflector, and classified according to type and frequency by the URBS PDP 11/04 computer and Tektronix 5L4N Spectrum Analyzer. Simultaneous recording of vocalizations, direct observations, and computer records of rat activity have been made to determine the types of vocalizations that are associated with specific behaviors. This vocal-ethogram library will be utilized to determine if any experimentally induced changes in vocalization and associated behavior occurred.

Correlation of the three types of data requires extensive cross referencing of very large computer files. In addition, those data sets that consist of direct visual observations of discrete behaviors, combinations of behaviors, and sequences of behaviors must be carefully indexed and stored in computer files before they can be matched to the vocalization and movement data which are already stored in separate computer files. Various methods of data presentation and analysis are being utilized to compress a very large amount of highly complex and diverse data into a simplified, quickly understood form.

Major Findings: Free ranging rats use ultrasonic vocalizations in several circumstances: by both dominant and subordinant in aggressive and agonistic interactions; during and following copulation; during brief, non-aggressive social interactions; and perhaps to indicate presence or even mood when no obvious social interactions are occurring. Of particular interest is the finding that these vocalizations may occur in patterns that we have called "conversations" during periods when little or no overt social behavior has been observed. These vocalizations occur at different but relatively close frequencies and are both simultaneous (which is clear evidence that more than one rat is vocalizing at the same time) and in sequence at different frequencies (which suggests that more than one rat is communicating). Our latest method of data examination indicates that as many as three different rats may have uttered ultrasonic vocalizations simultaneously. The determination of the exact social significance of such vocalizations awaits further analysis and examination.

Future course: To complete cross-referencing and analysis of our data files so that a comprehensive vocal/behavioral catalogue can be compiled.

Significance to Biomedical Research and the Program of the Institute: Vocal communication, which plays a significant role in human social behavior, may become changed as an outward manifestation of stress or some altered brain function. We present here an animal model which is being used to understand how long-term stress influences vocal communication, and which will be used to investigate the role of the neocortex in the regulation of vocal communication.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00838-04 LBEB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Theoretical Population Dynamics of Humans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John B. Calhoun	Research Psychologist	URBS LBEB NIMH
Other:	Dilip R. Ahuja	Fellow	HU

COOPERATING UNITS (if any)

Harvard University Interdisciplinary Program in Public Health

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

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NIMH, ADAMHA, NIH Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The trend of continuous increase in human world population characterizing the past two millenia, underwent a phase shift in 1975 to a slower rate of increase. Peak world population will be attained in 2065 and there will follow a decline to the 1975 level by 2175. Thereafter, decline will continue at an ever slower rate. Continuation of historical trends of population increase projects to an instantaneous increase to infinity. For the world this time varies between 2017 and 2026 A.D. Five major sets of regions of the world are characterized by the dates 1982, 1988, 2036, 2054 and 2140 when problems related to population density will become particularly critical.

PHS-6040
(Rev. 10-76)

Project Description:

Objective: To determine how change in rates of human population increase in each of 5 major sets of regions of the world interact and summate to produce a world trend in which each doubling of population takes half the time of the prior doubling.

Methods: The current year's project (a critical aspect of a longer range objective to develop an evolutionary model of population change -- past and future) utilized as a data base the McEvedy and Jones "Atlas of World Population History." Subsidiary populations were aggregated into 5 sets, S I to S V on the basis of one or more of the following: geographic contiguity, cultural similarity, and similarity of the exponential rate of increase of the Population Growth Rate (PGR) in recent times (the past 75 to 300 years). Best fit hyperbolic equations were calculated for 2 to 4 major time periods for each set over the past 2,000 years. For each equation the value, D_1, the year before continuation of prior trend culminates in an infinite population, was calculated.

Major Findings: 4 of the 5 sets exhibited 2 shifts in rate of change in PGR, and consequent patterns of population increase. One set had only one shift. Yet these fluctuations among sets interact to produce an overall world pattern of increase of population over time unmarked by temporal transitions in population dynamics. The world had a D_1 date of 2017 A.D. The D_1 of the 5 sets is listed below and shown in Fig. 1, rank-ordered by D_1 - 1975, where 1975 is the year for the world as a whole that population increase first declined below the historical trend of the past 2,000 years. The D_1 in Table I apply to the modern trend of population change.

Table I

Set	Inception date of modern trend	D_1	D_1 - 1975	Sets of Regions
I	1850	1982	7	China
V	1750	1988	13	Africa, South Asia, and Near East
III	1700	2036	61	Latin Europe, Eurasia, and Eastern Orient
IV	1800	2054	79	Latin America and Southeast Asia
II	1900	2140	165	Non-Mediterranean Europe and North America.

Fig. 1

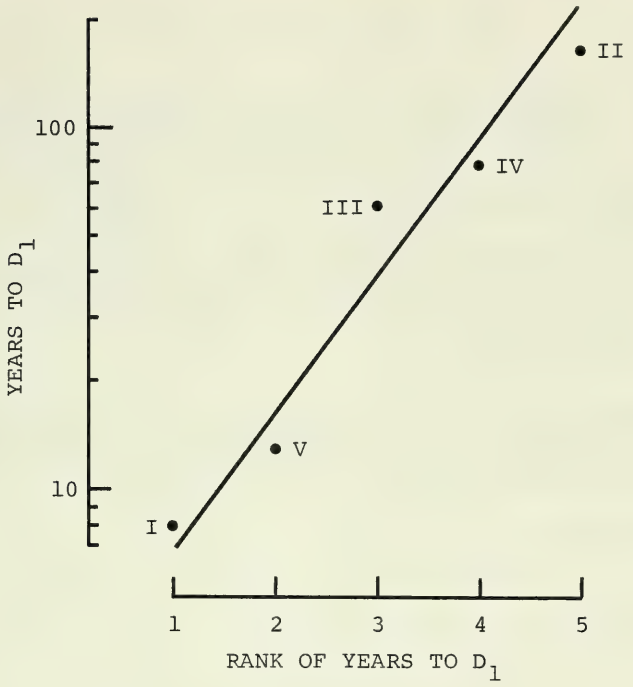
100
10
YEARS TO D_1
I
V
III
IV
II
1
2
3
4
5
RANK OF YEARS TO D_1

Fig. 2

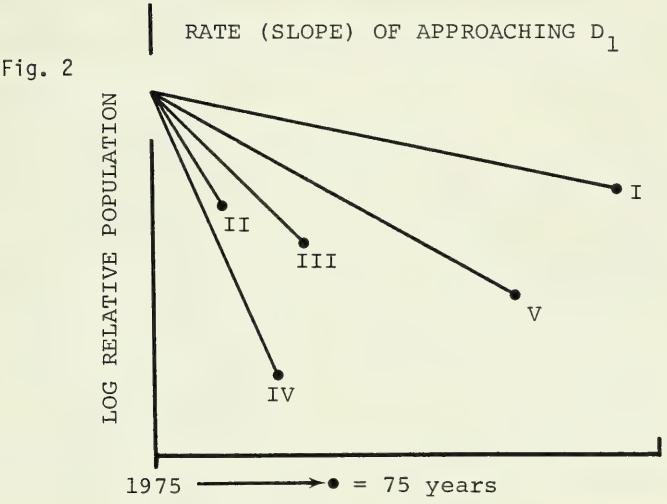
RATE (SLOPE) OF APPROACHING D_1
LOG RELATIVE POPULATION
I
II
III
V
IV
1975
= 75 years

Fig. 2 shows the rate that each Region approaches its own D_1, i.e.,the year before the historical hyperbolic increase can with certainty no longer hold. In general it may be said that the reciprocal of the slope of the line reflects the evolutionary vitality of a Set in the sense that the reduction of population growth in it reduced crowding in physical space, and increased conceptual space (the total pool of information capable of codification into useful ideas). The farther away a Set is from its own D_1, the greater should be the pressure from other Sets with lesser years to their D_1 for their residents to utilize both the physical and conceptual space of the Set with the later D_1.

Significance to Biomedical Research and the Program of the Institute: Our overall theory, including the present year's effort, provides a perspective for understanding the past course of enhancements of human welfare, as well as alerting us about the time course of likelihood of origin of problems related to population change.

Future Course: After completing this specific inquiry it is now possible to structure the final paper on the "Evolutionary World Population Model". The initial model was first presented at the 1968 Meeting of the AAAS as one of three invited "Moving Frontiers of Science" lectures.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00843-03 LBEB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Conceptual Prosthesis to the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John B. Calhoun	Research Psychologist	URBS LBEB NIMH
Other:	Garrett A. Bagley	Computer Programmer	URBS LBEB NIMH
	Etta Maye Zoerb	Editorial Assistant	URBS LBEB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

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NIMH, ADAMHA, NIH Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project had its roots in the practical problem of how to index literature data bases while preserving the authors' terminology. Each author often utilized two or more words, with overlapping meaning, to convey intent. Several thousand such pairs of words were subjected to computer analysis to determine the network character of their relationships. Their linkages formed a 3-dimensional network. Words near the center connoted control of response to assure survival. Outward from the center, words differentiated into ever more complex meanings through 7 steps of transition. Our model building led to three assumptions: (1) Each step in complexity of meaning represents an evolutionary advance in capacity for awareness and response accompanied by changes in brain function. (2) Words representing the nodes of this network reflect preverbal capacities of mammals to be aware of moods and emotions associated with experiencing day-to-day events. (3) Such awareness represents primitive conceptualization. As a specific modeling exercise we examined the inner core of the network, the portion possibly characteristic of primitive extinct mammals (see Project No. Z01 MH 00847-01). By this model, such a mammal has a potential awareness for over 5,000 experientially based moods and emotions.

Project Description:

Objective: To examine our model of the network of linkages between the meanings of words to determine what kinds of words might reflect the moods and emotions resulting from day-to-day experiences of primitive, extinct mammals.

The specific objective of this year's effort derives from a 15-year inquiry into the extensive network resulting from consideration of shared meaning between words. Since there are 7 levels of differentiation from the center of the network, and since more central terms seemed biased toward implication of being related to behaviors enhancing individual and species survival, it was hypothesized (1) that the outward differentiation reflected evolutionary differentiation in capacity to be aware of experience, and (2) that more centrally located words would provide some insight into the character of experience of primitive mammals.

Methods: Special computer programs were written and used to condense a larger set of words into 242 supra-generic concept sets, and to convert the network from 3 to 2 dimensions. Then the inner fourth (55 words) of the network was examined to produce a first approximation of the scope of affective awareness of primitive mammals. An examination of the within paragraph associations among these 242 supra-generic terms in a 300,000-word text revealed a high association of a word with its positionally polar opposite word. Therefore, after excluding the centermost word, the remaining 54 core supra-generic words were organized into 27 polar opposite pairs (Table 1).

Major Findings: As shown in Table 1, the polar opposite pairs of words fell into sets and subsets. For example subset A2 suggests a quite different aspect of behavior and experience than does subset B2. Furthermore, the 3 words selected to represent each of sets A, B and C provide insight into the basic orientation of primitive mammals. This can be phrased as the "attainment of goals, maintenance of wholeness, and expression of behavioral conformity". Likewise every pair of polar opposites may be expressed as a sentence, e.g.; A lc as "Attaining goals requires following pathways." Deriving 27 such statements from Table 1 may help to define key sets of behaviors most likely to distinguish differences between normal rats and those with chemically induced inhibition of neocortical development utilized in project Z01 MH 00847-01.

Significance to Biomedical Research and the Program of the Institute:

This exercise in model building, after 15 years of development, has still attained only a rudimentary level of clarification. However, it holds promise on both theoretical and practical grounds. On theoretical grounds, it suggests that there is a grammar to affect, which at the human level has verbal expression. Particulars of this expression seem to reflect various levels of affect spanning the mammalian lineage. If this is so, the analysis of verbal passages should be able to catagorize individuals along a spectrum of primitive to advanced affect orientation that mirrors the Maslow type of concern with level of development. On the practical side, permutated associations of supra-generic terms,

TABLE 1

Words as indicators of those behavioral processes which result in those moods or emotions that represent experiential conceptualization by primitive animals.

Network location code	Causal, positive or subject implication	Consequence complementarity or predicate implication	Set orientation by word representation
A 1a	COMMITMENT	ACCEPTANCE	
1b	VALUE	RULE	
1c	GOAL	PATHWAY	
A 2a	ethic	guidance	GOAL
2b	meaning	governance	choice
2c	awareness	choice	power
A 3a	optimum	perspective	
3b	analysis	embodiment	
3c	future	power	
B 1a	AUTHORITY	CONSERVATION	
1b	ACQUISITION	DEFENSE	
1c	WHOLENESS	RESTRAINT	
B 2a	structure	survival	WHOLENESS
2b	causation	preservation	adequacy
2c	promotion	inadequacy	integration
B 3a	integration	avoidance	
3b	information	inactivity	
3c	synthesis	crisis	
C 1a	FIXATION	INVARIANCE	
1b	EXPECTATION	STABILITY	
1c	CONFORMITY	CONTROL	
			CONFORMITY
C 2a	capacity	limitation	fitness
2b	potential	loss	responsibility
2c	fitness	design	
C 3a	adaptation	niche	
3b	success	responsibility	
3c	plan	image	

Notes:
1. A, B, C. represent major cores through the center of the network.
2. A1, B1, C1 (shown in CAPS) reflect the most basic central experiential processes.
3. Causal and consequence indicating words lie in polar opposite positions in the network.

indexed to paragraphs within a complex literature data base, have proven useful in identifying paragraphs that share objectives or concerns.

This annual project report assumes more significance in context with our earlier work (pp 1-187 in Physiological Mammalogy, Academic Press, 1963) where it is shown that the more advanced a mammal is in evolutionary terms, the more it inhibits the use of space by lesser advanced species. These earlier results, paired with the present line of inquiry, suggest that mammalian brain evolution permits individuals to cope with greater complexity of experiences on both intra- and interspecific levels as mammalian communities develop more role or niche complexity.

Future Course: FY 82 will be devoted to editorial efforts culminating in publication of the 300,000-word manuscript mentioned above in "Methods Employed".

Publication: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00847-01 LBEB NEW

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Role of the Neocortex in Coping with Complexity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James L. Hill	Visiting Associate	URBS LBEB NIMH
Other:	Garrett A. Bagley	Computer Programmer	URBS LBEB NIMH
	Charles L. Bishop	Biologist	URBS LBEB NIMH
	Etienne T. Lamoreaux	Psychology Technician	URBS LBEB NIMH

COOPERATING UNITS (if any)

None

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Laboratory of Brain Evolution and Behavior

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	0.9	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The offspring of female rats injected during pregnancy with Methylazoxymethanol acetate (MAM) develop a markedly reduced cerebral neocortex. This chemically induced micrencephaly does not appear to severely alter normal rat behavior patterns since similarly treated animals are capable of learning, reproduce successfully, and may exhibit many other species typical behaviors. However, these behaviors have been observed only in relatively restricted laboratory cage environments which may present no real challenge to the survival of a micrencephalic individual. It is the purpose of this study to examine the behavior of micrencephalic rats living as free ranging groups in large, complexly structured environments. It is anticipated that, initially MAM-treated animals will exhibit behavioral deficits and may not be capable of fully utilizing the complex environments until long after normal animals, thereby indicating that one of the roles of the neocortex is to facilitate the rapid, full utilization of new and complex environments.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine if rats require all of the neocortex to perform appropriate on-going behaviors to survive for extended periods living as groups in complex environments.

Methods: On day 15 of pregnancy, female rats are injected with a 30 mg/Kg dose of Methylazoxymethanol acetate (MAM); this chemical inhibits the multiplication and associated migration of neuroblasts in the developing neocortex of the fetus and, thereby, prevents the full development of the neocortex. Individual and social behaviors of small populations of micrencephalic rats (from MAM injected females) will be compared to those of controls (from saline injected females) in different environments, individual learning tasks, and social situations.

The animals will be housed in large experimental rooms of two distinct levels of complexity, and in two types of cage providing distinct levels of internal structural complexity. Group size will also be varied among the larger groups housed in the more complex environments. Movement patterns of animals in the rooms will be monitored by a computer controlled data acquisition system, and behavioral interactions will be recorded during direct visual observations. Recordings of ultrasonic vocalizations from direct on-going activity will be made. Data related to reproductive, physical, and physiological conditions will be recorded during periodic surveys of all animals. Tests of learning capacity will be conducted both during discrete test situations and during normal, on-going activity.

Major Findings: The offspring of female rats injected with MAM on day 15 of pregnancy exhibit many species typical rat behaviors despite their micrencephalic condition. Of particular interest are social behaviors that have been performed in the typical manner and in appropriate circumstances. These include elements of social and sexual grooming and investigation, and social play behavior. That micrencephalic rats are also capable of admitting ultrasonic vocalizations is a finding, thus far, unique to this laboratory. The degree of similarity to normal rat ultrasonic vocalization awaits further study.

Future Course: To closely monitor the activities of rats to determine if any behavioral deficits attributable to micrencephaly are lessened by the experience of living in complex environments.

Significance to Biomedical Research and the Program of the Institute: Extensive, experimentally induced micrencephaly apparently results in only minor behavioral deficits in rats. However, the behavior of rats with substantial losses of neocortex has only been observed within the restrictions of a few, limited experimental tests. The full repertoire of behaviors that may be required for the successful exploitation of complex environments, within the context of on-going social interactions, has not been examined. This project, in which the degree of neocortical development in rats can be limited, represents a model system for determining how a micrencephalic organism deals with complex environments and social situations. This research will also permit a determination of the degree to which species typical behaviors that are controlled by

lower brain centers may be regulated by so called higher centers in the neocortex.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00881-25 LCM

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Intermediary Energy Metabolism in Mammalian Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. E. Kaufman	Research Chemist	LCM NIMH
OTHER:	T. Nelson	Staff Fellow	LCM NIMH
	L. Sokoloff	Chief, Lab. of Cerebral Metabolism	LCM NIMH

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None

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.75	2.0	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project continues into its second year of studies on the origin, mode of action, and metabolism of γ-hydroxybutyrate, (GHB), a compound which is a normal constituent of mammalian brain and which is of increasing pharmacological interest. When administered in pharmacological doses, GHB produces a flattening of the EEG, a reversible trance-like state and a profound depression of cerebral glucose utilization. This compound is being used clinically in Europe as an anesthetic adjuvant. Preliminary trials indicate that it is effective in the treatment of narcolepsy and may be of use in the treatment of stroke.

An enzyme capable of metabolizing GHB has been found in tissues such as liver and kidney as well as in brain. This enzyme, an NADP$^+$-linked alcohol oxido-reductase which interconverts GHB and succinic semialdehyde, has been purified from hamster liver and partially purified from hamster brain. Studies completed on this enzyme include substrate specificity, enzyme kinetics, molecular weight determination, and inhibition studies with the anticonvulsant agents, diphenylhydantoin, amobarbital, and valproate.

PHS-6040
(Rev. 10-76)

Project Description:

This project is directed at understanding the role of naturally-occurring γ-hydroxybutyrate as well as the metabolic pathways involved in its synthesis and degradation. Since the compound is also used as a drug, other important questions arise concerning its pharmacological actions. In addition to the pharmacological effects already mentioned (flattening of the EEG, trance-like state and depression of cerebral glucose utilization) it has recently been reported that γ-hydroxybutyrate increases the survival of animals which have been made hypoxic. The authors report that the blood and brain tissues of animals which have received γ-hydroxybutyrate showed marked changes in the levels of some intermediates in carbohydrate metabolism as well as changes in levels of high energy phosphate compounds. These studies have led to the proposal that γ-hydroxybutyrate may be useful in the treatment of stroke. Because γ-hydroxybutyrate is a compound which is used clinically, and because it is a naturally-occurring compound, we feel that it is important to understand not only pathways of metabolism of γ-hydroxybutyrate, but also the factors which control these pathways.

We have recently found that an oxido-reductase which catalyzes the oxidation of GHB also catalyzes the reduction of D-glucuronate to L-gulonate. The reduction of D-glucuronate to L-gulonate is an important step in the pathway leading from UDP-glucose to L-ascorbate or to L-xylulose and the pentose pathway. We have recently investigated the effect of both L-gulonate and of D-glucuronate on the rate of oxidation of γ-hydroxybutyrate. In the presence of limiting concentrations of $NADP^+$ or with limiting concentrations of $NADP^+$ and inhibitory concentrations of NADPH, D-glucuronate can increase the rate of oxidation of GHB by up to eightfold. Since this enzyme is a soluble enzyme found in the cytosol and since the cytosol of most tissues contains low concentrations of $NADP^+$ as well as relatively high concentrations of NADPH, D-glucuronate may be an important factor in controlling GHB metabolism *in vivo*.

The physical and chemical properties of this oxido-reductase are also being investigated. Treatment of the enzyme with a reducing agent such as dithiothreotol produces marked inhibition of activity. This inhibition can be at least partially reversed by the addition of an oxidizing agent such as hydrogen peroxide. We have recently found that complete reversal of the inhibition by dithiothreotol can be achieved by the addition of oxidized glutathione. This suggests that the activity of this enzyme may be regulated by the ratio of oxidized to reduced glutathione. We are attempting to correlate this effect with the effect of these compounds on the physical state of the protein.

A study on the purification and characterization of this oxido-reductase has been completed and published. This enzyme has been purified 300-fold and found to exhibit a single band on gel electrophoresis. The protein is a monomer with a molecular weight of ∿ 31,000. The enzyme is inhibited by two anticonvulsant agents, amobarbital and diphenylhydantoin, as well as by valproate, a drug used in the treatment of epilepsy. Pyrazole, the specific inhibitor of NAD^+-linked alcohol dehydrogenase (ADH), does not inhibit this enzyme. Diethyldithiocarbamate, the reduced form of the drug antabuse, as well as KCN, both metal-chelating agents, inhibit the enzyme. This suggests that the enzyme may contain either copper or zinc as an integral part of the protein. This possibility is currently being investigated.

ADH is also capable of catalyzing the interconversion of γ-hydroxybutyrate and succinic semialdehyde (SSA) in vitro. The Km for γ-hydroxybutyrate with alcohol dehydrogenase is, however, an order of magnitude higher than the Km with the $NADP^+$-linked oxido-reductase. In vivo studies on the half-life of [^{14}C]γ-hydroxybutyrate (in plasma) in the presence and absence of various inhibitors have been carried out in an effort to assess the contribution of these two enzymes to the metabolism of γ-hydroxybutyrate in the whole animal. Results of these studies show that pyrazole, a potent inhibitor of ADH, does not affect the half-life of γ-hydroxybutyrate. The same concentration of pyrazole produced severe inhibition of ethanol metabolism in the whole animal. This suggests that ADH is probably not the enzyme responsible for the oxidation of γ-hydroxybutyrate in the whole animal. Preliminary results show that two compounds which inhibit the oxidation of γ-hydroxybutyrate catalyzed by the $NADP^+$-linked oxido-reductase, dilantin and L-gulonate, increase the half-life of γ-hydroxybutyrate in the plasma (indicating a decreased rate of metabolism). These results lend support to a role for the $NADP^+$-linked alcohol oxido-reductase in the metabolism of γ-hydroxybutyrate in the whole animal. Recent evidence suggests that this enzyme is not involved in the synthesis of GHB but rather in its degradation.

Antibody. The purified $NADP^+$-linked alcohol oxido-reductase (γ-hydroxybutyrate dehydrogenase, which interconverts γ-hydroxybutyrate and succinic semialdehyde, has been used to raise antibody in New Zealand white rabbits. This antibody will be purified and used to confirm the role of the alcohol oxido-reductase as a degradative enzyme for γ-hydroxybutyrate. The antibody will also be used to determine whether the γ-hydroxybutyrate dehydrogenase from brain and extraneural tissue are indeed the same enzymes.

GHB levels in organs. The survey of rat organs for γ-hydroxybutyrate has been completed and the results are in the process of being published.

Gamma hydroxybutyrate is widely distributed throughout all the organs which have been examined; its concentration in rat organs is shown in the accompanying table. Concentrations are expressed in nanomoles per gram of tissue.

ORGAN	BRAIN	HEART	KIDNEY	LIVER	LUNG	MUSCLE	BROWN FAT	WHITE FAT	BLOOD
Mean GHB	2.29	12.4	28.4	1.4	1.5	10.2	37.4	0.42	0.55
Std.Error	0.13	1.9	4.6	0.3	0.2	1.6	2.1	0.27	0.27
Number	36	36	36	36	36	36	100	7	12

These findings suggest that γ-hydroxybutyrate plays some role in many, if not all, of the organs in the body. Until those results were obtained, it had been assumed that the most likely function of γ-hydroxybutyrate was to modulate dopaminergic neuronal activity. Now additional functions will have to be sought for γ-hydroxybutyrate in non-neural tissues, and these new functions may also have relevance to the brain. The most likely precursors for γ-hydroxybutyrate were thought to be glutamic acid, γ-aminobutyric acid, and succinic semialdehyde, since experimental evidence shows that each of these molecules is capable of contributing carbon atoms to the skeleton of γ-hydroxybutyrate. The finding of high levels of γ-hydroxybutyrate in tissues such as kidney, which lack the means of decarboxylating glutamate to form γ-aminobutyrate, suggests that there may be other precursors for γ-hydroxybutyrate than those which have been identified.

Hibernation. The hibernating golden Syrian hamster has been surveyed for the concentration of γ-hydroxybutyrate in brain, kidney, liver, muscle, brown fat and white fat. The observation which led to this investigation was that exogenously administered γ-hydroxybutyrate produced a behavioral state in which rats became unresponsive to stimuli, dropped their body temperature and respiratory rates. These effects appeared reminiscent of the findings in hibernating animals. It was anticipated that the brain γ-hydroxybutyrate levels would be higher in the hibernating hamster than in the awake warm or cold controls; however, there was no difference between the brains of the hibernating or control hamsters. In two organs, the kidney and brown fat, the levels of γ-hydroxybutyrate were markedly higher than the controls. It appears that the hibernating hamster may serve as a useful model for studying physiological events which modify the synthesis and degradation of γ-hydroxybutyrate. Further studies using this model are in progress.

A new spectrophotometric assay for the detection of γ-hydroxybutyrate has been devised. It is based on the use of the purified $NADP^+$-linked alcohol oxidoreductase and the synthetic co-factor, 3-acetylpyridine-adenine dinucleotide phosphate. The substitution of the synthetic co-factor shifts the equilibrium of the reaction GHB + $NADP^+ \rightleftharpoons$ SSA + NADPH + H^+ far to the right so that the GHB may be estimated by the amount of reduced co-factor which has been produced. We anticipate that this assay will prove useful in the estimation of blood and tissue levels of γ-hydroxybutyrate.

A study has just been completed in which we have determined both GHB levels and soluble cytosol $NADP^+$ linked GHB dehydrogenase activity in developing rats from the late fetal stage to 20 postnatal drugs. GHB concentrations in the newborn rat brains and livers are two to three times higher than they are in the adult. The concentration of GHB gradually decreases over the first 20 days of life to adult levels. GHB levels in the kidney rise from a lower level in the newborn period to attain the high level characteristic of the adult by 20 days. The enzymatic activities, on the other hand, tends to increase from the time of birth to 20 days in all the tissues, including brain.

This pattern of developmental changes is of special significance since previous widely accepted studies by other workers have suggested that GABA is the precursor for GHB in the brain. A soluble cytoplasmic enzyme has been implicated in the reduction of succinic semialdehyde in the cytosol to form GHB. It is known that during the same period of development in the rat both GABA transaminase and glutamate decarboxylase activity are low at birth and increase by some tenfold. These findings are consistent with a rate of GABA production which is low in the newborn and increases with age while our present study shows GHB, a putative metabolic derivative of GABA, is highest at a time when GABA synthesis is low and falls as the rate of GABA synthesis increases.

These findings cast doubt on the widely accepted notion that GABA is the main precursor for GHB in the brain, at lease in the newborn period. The discovery of considerable concentrations of GHB in extraneural tissues and the observation of the discrepancy between GABA and GHB concentrations in the developing brain has caused us to look for other possible precursors for GHB.

One of the new aspects of this project is an effort to work out the biosynthetic pathway for GHB. We have used three main approaches to this problem. In

the first, a number of carbohydrate intermediates, amino acids, and fatty acids were tested in an in vitro system for the ability to produce net synthesis of GHB. This has been a general survey of a number of possible precursors for GHB.

The second approach was similar to the first except that radioactive compounds were used in order to determine whether the compounds which gave rise to GHB in the first part actually contribute carbon atoms to the GHB molecule.

The third approach will be in vivo experiments in which a radioactive precursor is injected and GHB is then isolated from the various tissues to determine whether has been incorporated in the GHB pool.

Preliminary results from the general survey (in vitro) indicate that both palmitic acid and DL-β-hydroxybutyrate are capable of stimulating the formation of GHB. This pathway is located in the mitochondrion and is oxygen-dependent.

This project will have a continued interest in ketone body metabolism in normal as well as in pathological states. D-β-Hydroxybutyric acid dehydrogenase catalyzes the reversible interconversion between D-β-hydroxybutyrate and acetoacetate, the two ketone bodies found primarily in the liver when fatty acid utilization is increased, as, for example, in diabetes, starvation, high-fat diet, and various states leading to ketosis. Acetoacetate is the primary ketone body formed from catabolism, and it is converted to D-β-hydroxybutyrate by the action of the enzyme. Once formed, however, D-β-hydroxybutyrate cannot be used directly in any of the tissues; it must first be converted back to acetoacetate by the action of the same enzyme. This raises the interesting question of what role the enzyme plays. Why does the body not utilize the directly-formed acetoacetate rather than convert it first to D-β-hydroxybutyrate only to oxidize it back to acetoacetate again to utilize it? There is evidence in the literature to suggest that excessive concentrations of acetoacetate in the tissues may be toxic, particularly in brain which has recently been shown to utilize ketone bodies in direct proportion to their levels in the blood. The possibility existed that the role of the enzyme is protective to keep the acetoacetate concentrations low by storing it, in a sense, as D-β-hydroxybutyrate. It is noteworthy that there are several metabolic conditions characterized by ketosis and coma. Diabetic coma is one example, and recently Reye's Syndrome has been described. This is a frequently fatal disease of childhood which occasionally follows a viral infection, particularly influenza. It is characterized by fatty degeneration of the liver, ketosis, and coma.

Examination of the metabolic pathways and the enzymes responsible for the metabolism of ketone bodies in brain suggests possible mechanisms by which forced excessive use of ketone bodies in brain may lead to depressed cerebral functional activity. The ketone bodies enter the Krebs Cycle after their prior conversion to acetylCoA. The pathway is the conversion of β-hydroxybutyrate to acetoacetate which then displaces succinate from succinylCoA to form acetoacetylCoA. The acetoacetylCoA is then converted to acetylCoA and enters the Krebs Cycle. For every acetoacetate molecule thus metabolized, there is one succinylCoA degraded. SuccinylCoA is required for the formation of GTP which is the precursor of cyclic GMP. Excessive use of ketone bodies can theoretically, therefore, lead to depletion of available GTP and, therefore, also cyclic GMP. There is now a considerable body of evidence that cyclic GMP is the second messenger in the action of the neurotransmitter, acetylcholine. Its depletion can be expected to result in depression of

cholinergic functions in brain and lead possibly to depressed functional activity and even coma. The possibility that ketosis may lead to coma by depletion of brain cyclic GMP is currently being studied in this laboratory. A radioimmune assay for cyclic GMP has been established and preliminary results do indeed suggest that in diabetic acidosis there is a depression of cyclic GMP in brain.

Proposed Course:

This project will continue on the course which has been outlined. It will include: 1) further kinetic studies with $NADP^+$-linked alcohol oxido-reductase; 2) determination of the presence or absence of a metal in this enzyme; 3) in vivo studies to determine the effect of various drugs, normal metabolites and alterations in physiological state on the metabolism of GHB; 4) continued investigation of GHB metabolism in the hibernating animal; 5) investigation of the interaction of GHB with other pathways of carbohydrate metabolism; 6) study of the biosynthetic pathway for GHB; 7) developmental studies on the levels of GHB and of the $NADP^+$-linked alcohol oxido-reductase in brain and other tissues. 8) This project will have a continued interest in ketosis, β-hydroxybutyrate metabolism and the relationship between ketosis and cyclic GMP levels in brain.

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Kaufman, E.E., and Nelson, T.: Kinetics of coupled γ-hydroxybutyrate oxidation and D-glucuronate reduction by an $NADP^+$-dependent oxido-reductase. J. Biol. Chem. 256(13): 6890-6894, 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00882-14 LCM

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
Studies on Regional Cerebral Circulation and Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Sokoloff	Chief, Lab. of Cerebral Metabolism	LCM NIMH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A method has been developed for the quantitative determination of the rates of local glucose consumption in the discrete functional and structural components of the brain in conscious or anesthetized laboratory animals. The method is based on the use of [^{14}C]deoxyglucose as a tracer for glucose flux through the hexokinase step. Local [^{14}C]deoxyglucose-6-phosphate concentrations in the tissues of the CNS are measured by a quantitative autoradiographic method. Inasmuch as the autoradiographs of the relative rates of local glucose consumption can be used directly for mapping metabolically, and therefore functionally, linked structures in the CNS, the method is being used to study alterations in the energy metabolism of the discrete functional and structural components of the brain in a variety of physiological, pharmacological, and pathological states.

PHS-6040
(Rev. 10-76)

Project Description:

Previous work in this Laboratory led to the development of a method for the measurement of the rates of blood flow in the structural and functional units of brain in conscious laboratory animals. The method was based on the uptake of a radioactive, chemically inert gas into the tissues of the brain, and a unique quantitative autoradiographic technique was developed which made possible the measurement by densitometric procedures of the concentrations of the radioactive tracer in the individual structures of the brain down to a resolution of 0.2-0.5 millimeters. The key to the fine resolution of the method was the autoradiographic technique.

Although measurement of local cerebral blood flow is inherently interesting with respect to the physiology, pharmacology, and pathology of the circulatory system, it is of limited value in studies of cerebral functional and biochemical activity. The Laboratory, therefore, addressed itself to the development of a method to measure local cerebral energy metabolism with the same degree of structural resolution because energy metabolism could be expected to relate more closely to local cerebral functional activity. It was always anticipated that the quantitative autoradiographic technique designed for the blood flow method would also be at the heart of such a method. It was necessary, however, to choose an appropriately labeled precursor of cerebral energy metabolism. Oxygen could not be used because there are no suitable radioisotopes of oxygen. [^{14}C]Glucose also appeared to be unsuitable because glucose is too rapidly metabolized, and its radioactive products are too quickly removed from brain. It was, therefore, decided to use [^{14}C]deoxyglucose, an analogue of glucose which is handled qualitatively just like glucose by the transport system in the blood-brain barrier and by the initial enzyme, hexokinase, in the pathway of glucose metabolism. Once phosphorylated, however, the deoxyglucose is trapped, unlike glucose which is metabolized further to carbon dioxide and water. Quantitatively, however, deoxyglucose phosphorylation and glucose phosphorylation or utilization are different inasmuch as the transport carrier and the enzyme discriminate kinetically between the two substrates. It appeared to be a simple matter to apply the autoradiographic technique to measure deoxyglucose phosphorylation, but to relate it to the steady state rate of glucose flux through the phosphorylation step, which is a measure of the rate of glucose consumption, required the solution of numerous theoretical and technical problems.

A theoretical model, which encompassed all that we knew about deoxyglucose and glucose transport between brain and blood and their metabolism in brain tissue, was constructed, and mathematical relationships to describe the model were developed. Experiments were done on one point or another to evaluate and, if necessary, to revise the model and the mathematical relationships to fit the model closer to the real situation.

It was clear from the model that to determine the rate of glucose consumption from the rate of [^{14}C]deoxyglucose phosphorylation would require the determination of the distribution volumes of deoxyglucose and glucose in the cerebral tissues and the hexokinase kinetic constants (V_{max} and K_m) for both deoxyglucose and glucose. By appropriate mathematical manipulations, it was possible to segregate all these separate constants into a single "lumped constant" encompassing all of them. It was now necessary to determine only the single lumped constant rather than the six

individual ones. Further mathematical analyses revealed the way to design an experiment to determine the "lumped constant". Another equation was developed from the model which showed that if the arterial concentration was maintained constant for a sufficient length of time, e.g., at least 20 minutes, then the ratio of the cerebral extractions of deoxyglucose and glucose would reach a constant level equal to the lumped constant. With the help of the Theoretical Statistics and Mathematics Branch, it was found possible to derive from the analyses of plasma disappearance curves of deoxyglucose an intravenous infusion schedule which results in a constant arterial deoxyglucose concentration for up to 45 minutes or longer. Surgical procedures were developed in the rat, monkey, and cat to sample arterial and cerebral venous blood from which the extraction ratios are determined. The lumped constant has been fully determined in the conscious and anesthetized rat; its value is 0.483, and it is unchanged in a variety of physiological and pharmacological states. The lumped constant has recently been determined in the monkey and the cat; the values have been found to be 0.344 and 0.41, respectively. In collaboration with T. Duffy of the Department of Neurology, Cornell University, the lumped constant has been measured in the dog and found to be 0.56.

All the theoretical and technical problems were solved, and the method has now been completely operative for the last four years. An equation has been derived which relates the rate of glucose consumption to measurable variables and allows the calculation of glucose consumption in the discrete structural and functional units of the brain. The equation prescribes the procedure to be used and the variables to be measured. An intravenous pulse of [^{14}C]deoxyglucose is injected, and arterial plasma concentrations of [^{14}C]deoxyglucose and glucose are measured from the time of injection till 30-45 minutes when the animal is decapitated, and the head frozen. Sections of brain are prepared from which local cerebral tissue [^{14}C]deoxyglucose concentrations are determined by the quantitative autoradiographic technique. From these measured variables and the lumped constant, local cerebral glucose utilization is calculated by the equation. The procedure for calculation has been programmed, and all the calculations are carried out by a computer.

The regional localization obtained with the [^{14}C]deoxyglucose method is achieved by the use of quantitative autoradiography. The autoradiographs provide pictorial representations of the relative, not the actual, rates of glucose utilization in all structures of the brain. They are ordinarily subjected to manual densitometric analysis from which local ^{14}C concentrations are derived and used in the operational equation to compute the actual rates of local glucose utilization. We have recently developed a computerized image-processing system to analyze and transform the autoradiographs into color-coded pictorial maps of the actual rates of glucose utilization throughout the entire CNS. The autoradiographs are scanned automatically by a computer-controlled scanning microdensitometer which measures the optical density of each spot, 25-100 μm, in the autoradiograph. These optical densities are stored in a computer, converted to tissue ^{14}C concentrations on the basis of the optical densities of calibrated ^{14}C plastic standards, and then converted by the computer to actual local rates of glucose utilization by solution of the operational equation. Colors are assigned to narrow ranges of the rates of glucose utilization, and the autoradiographs are then displayed on a color monitor in color along with a calibrated color scale for identifying the rate of glucose utilization in each spot of the autoradiograph from its color. These pictures are,

therefore, complete color maps of the actual rates of local glucose utilization precisely localized in each 25-100 μm region of the CNS. Work is in progress to develop computerized techniques to reconstruct color maps of the entire brain three-dimensionally from the digitized autoradiographs.

The [^{14}C]deoxyglucose method has been applied to normal conscious and anesthetized rats and conscious monkeys. The values obtained cannot be directly compared to any other data in the literature since measurement of local cerebral glucose consumption has never previously been possible, but the average of the local values is very close to the reported values for overall average brain glucose utilization in those two species. Anesthesia has been found to depress cerebral glucose consumption throughout the brain. Carbon dioxide inhalation has been shown to depress local glucose consumption even though it increases cerebral blood flow. Local cerebral blood flow has been found in the normal animal to correlate very closely with local cerebral energy metabolism, a relationship which had been hypothesized but never previously proved. Increased local functional activity produced by electrical stimulation or by experimentally induced focal seizures following local penicillin injection results in markedly increased local glucose consumption in appropriate regions of the CNS. The studies with experimental focal epilepsy have established the existence of a "mirror focus" contralateral to the epileptogenic focus. These results have established once and for all the often debated but never previously proved fact that energy metabolism in the CNS is closely coupled to functional activity as it is in all other tissues.

Studies have been completed on the effects of normal aging in rats from young adulthood of 4-6 months to old age of 26-36 months. The results demonstrate decreases in glucose utilization in selective regions of the brain. There is a general reduction in activity and the extent of the neuropile through most of the cerebral cortex. There are decreases in the caudate-putamen system. There is a decrease in the parietal cortex. The most prominent changes, however, are in the primary sensory systems, mainly those involved with vision and audition. These entire systems were markedly depressed metabolically, suggesting that there was a depression of sensory input due to peripheral sense organ failure, i.e., the eyes and the ears. Such results might suggest that major depressions in some brain functional activities may be the result of essentially deafferentations or separation from the environment because of functional losses in the peripheral sense organs. Studies in progress, in which dopaminergic systems are activated by administration of the dopamine-agonist, apomorphine, indicate age-related depression in responsivity of the nigrostriatal system, perhaps due to losses in dopamine receptors.

Studies have been completed on the effects of amphetamine in the rat. This dopaminergic agonist produces pronounced increases in glucose utilization in selected structures of the brain, in particular the components of the extra-pyramidal dopaminergic motor system, i.e., caudate nucleus zona compacta and zona reticulata of the substantia nigra, etc. A markedly affected structure was the subthalamic nucleus, which had previously not been suspected to be dopaminergic, but in follow-up studies based on those of this laboratory, has been found to contain an active dopamine-sensitive adenylcyclase. Similar studies have been carried out on the effects of another type of dopamine-agonist, apomorphine, which has been found to have similar effects on local cerebral glucose utilization. Studies have been completed in collaboration with Drs. Agu Pert and William Bunney of the Adult

Psychiatry Branch, NIMH, on the supersensitization of dopaminergic synapses by chronic administration of the dopamine-blocking drug, haloperidol. The results thus far clearly demonstrate metabolic correlates of the super-sensitivity phenomenon. Studies of the actions of apomorphine also led to the demonstration of dopaminergic synapses in the retina in the neural pathway from the retina to the superior colliculus.

Both dopamine-agonists, d-amphetamine and apomorphine, activated metabolism in all known dopaminergic structures except those of the mesolimbic system, which is a dopaminergic system suspected to be excessively active in amphetamine-psychosis and possibly also schizophrenia. The lack of effect of dopamine-agonists on this system remained, therefore, a puzzle. Studies during the last year, however, have demonstrated that the system is activated by chronic administration of d-amphetamine for as long as a week, a regime more like that associated with amphetamine-psychosis in man.

The method has also been used to study the visual systems of albino and pigmented rats. It has shown that the visual system of the albino rat is more sensitive than that of the pigmented rat at very low light levels but is overwhelmed or "fatigued" at higher light levels, which cause a reduction in local glucose utilization in the visual structures. These studies have also demonstrated a linear relationship between the rate of local glucose utilization in central visual structures and the log of the intensity of retinal stimulation, indicating that local metabolic rate in the nervous system, like behavioral manifestations, obeys the Weber-Fechner Law.

Studies on the superior cervical ganglion have demonstrated that the local rate of glucose utilization is closely coupled to the electrical activity. Electrical stimulation of the pre-ganglionic input to this ganglion results in increased glucose utilization in the ganglion, and the rate of glucose utilization is directly and linearly related to the spike frequency. Antidromic stimulation of the efferent nerves from this ganglion stimulates glucose utilization in the cell bodies of origin of the efferent nerve. The locations of the cell bodies are clearly visualized in the autoradiographs, and this combination of antidromic electrical stimulation with the deoxyglucose method offers promise as a technique to find the origin of neural pathways in the nervous system.

Studies are in progress to identify the central nervous mechanisms involved in the pathogenesis of the hypertension in spontaneous hypertensive rats. This is a genetic strain of rats in which malignant hypertension, which is believed to result from overactivity of the sympathetic nervous system, develops as the rats mature. Studies carried out in the hypertensive rats reveal increased glucose utilization only in the vestibular, fastigial, and cuneate nuclei. The activation of the cuneate nucleus is probably a consequence of the hypertension, inasmuch as it receives input from the baroreceptors that are stimulated by blood pressure. The significance of the changes in the fastigial and vestibular nuclei is uncertain. These nuclei are part of a pathway that adjusts blood pressure to changes in posture and could, therefore, be responsible for the hypertension. Destruction of these nuclei bilaterally has, however, failed to restore the blood pressure to normal. A surprising finding of these studies was that glucose utilization in the sympathetic ganglia (e.g., superior cervical, cardiac, and coeliac ganglia) was markedly reduced rather than elevated, which would be expected if, in fact, the

sympathetic system were overactive. These results, therefore, cast doubt on overactivity of the sympathetic nervous system as responsible for the hypertension. Studies are continuing to ascertain the cause of the low metabolic rate in the sympathetic ganglia.

Local cerebral glucose utilization has been measured in young adult monkeys during slow wave sleep. The results demonstrate that there is a diffuse generalized reduction of metabolic rate of 20-30% throughout the brain. No regions were found with increased glucose utilization, even those postulated to be sleep-centers which when activated produce the state of sleep. These studies cast doubt on the hypotheses concerning specific sleep centers in the brain.

The deoxyglucose method was originally designed for use in animals in a relatively normal physiological state. The lumped constant and the rate constants were all determined in normal animals. Experience with the method in pharmacological and pathological states indicated, however, that these constants did not always apply to the condition under study. In particular, severe hypoglycemia and hyperglycemia were conditions in which the lumped constant and rate constants were altered markedly from their values in normoglycemia. The lumped constant was redetermined, therefore, over the entire range of plasma glucose concentrations from 40-600 mg%. It does, in fact, change at the extreme limits of this range, but the appropriate values are now known. The rate constants over this same range are now being determined. When all these constants are finally known, the method will be applicable to conditions, such as stress, epinephrine infusion, etc., which alter the blood glucose concentrations beyond the normal range.

The deoxyglucose method was originally designed for use with quantitative autoradiography with ^{14}C. ^{14}C was chosen because of the availability of X-ray film sensitive to its β-radiations. The resolution of the method with ^{14}C is approximately 100 μm. Resolution could be improved to approximately 10 μm if [^{3}H]-deoxyglucose were used instead of [^{14}C]deoxyglucose. X-ray film (LKB [^{3}H]Ultrafilm) is now available for ^{3}H autoradiography, and studies are in progress to modify the original method for use with ^{3}H. It is necessary to calibrate [^{3}H]-methylmethacrylate standards to quantify the autoradiography; the calibration procedures are now in progress.

Recent technological developments with computerized axial tomography have made it possible to scan the human brain for local tissue concentrations of positron-emitting isotopes. Experiments have, therefore, been carried out with [^{18}F]-fluoro-deoxyglucose, a positron-emitting analogue of [^{14}C]deoxyglucose, and the results demonstrate that it can be used in place of [^{14}C]deoxyglucose as the tracer for the deoxyglucose method. This now makes it possible to use the method in man. In collaboration with Dr. M. Reivich of the University of Pennsylvania, and M. Phelps and D. Kuhl of UCLA, the method has been adapted for human use. The NINCDS has procured a scanner which is now operational. This instrument is available for our use, and we are collaborating with other groups in studies of psychotic states, aging, and dementia in man.

Several lines of further investigation are planned in this project. One line is methodological by which it is hoped to improve the resolution of the method to the microscopic or single cell level. Another line is the exploitation of the [^{14}C]deoxyglucose method at its present state in studies of the effects of aging,

psychoactive drugs, metabolic comas, development and maturation of the brain, and hormones such as thyroxine known to have important actions on the maturation of the brain.

APPENDIX

Contract: Contract No. 278-79-0004 (IR) University of Montreal
Contract Title: Deoxyglucose Method for Measurement of Local Cerebral Glucose Utilization

The present deoxyglucose method achieves a spatial resolution of 100 μm. This is adequate to measure glucose utilization only macroscopically in the structural components of the nervous system. For many neurophysiological and neuroanatomical studies it is necessary to study single cells or even components of cells. To achieve this requires extension of the resolution of the method to the light and electron microscopic levels. The theoretical basis of the method allows for such resolution, but technical problems associated with autoradiography of water-soluble tracers make this a difficult problem.

The Laboratory of Cerebral Metabolism lacks the physical facilities and resources as well as the necessary experience and expertise to carry out the necessary neuroanatomical procedures required for this technological development. A contract was, therefore, made with Drs. M.H. Des Rosiers and L. Descarries of the University of Montreal to undertake the studies needed to accomplish this development. Dr. Des Rosiers was trained in all the intricacies of the deoxyglucose method in our Laboratory; indeed, he was a member of the team that developed the method. Dr. Descarries directs a neuroanatomical laboratory which has had extensive experience with high resolution autoradiography. They have made considerable progress toward the achievement of microscopic resolution. They utilize [^{3}H]deoxyglucose and perfuse the brain *in vivo* with glutaraldehyde and osmic acid. They then remove the brain, dehydrate it with anhydrous acetone, and embed it in Epon. This procedure manages to preserve the labeled compound in the tissue without moving it from its original site. The embedded brain is then sectioned for autoradiography. It is necessary to use dipping emulsion for the autoradiography, but dipping emulsion is water-based and may wash out the label. Work is progressing on finding a technique by which the dipping emulsion will not displace the label. Progress is slow but steady. When this goal is achieved, the method should be close to becoming operational for studies at the cell and sub-cellular levels.

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October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)
The Extended Visual System of the Macaque Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Kennedy	Guest Worker	LCM NIMH
	M. Mishkin	Research Physiologist	LN NIMH
OTHER:	M. Ito	Visiting Associate	LCM NIMH
	S. Suda	Visiting Fellow	LCM NIMH
	K. Macko	Guest Worker	LN NIMH

COOPERATING UNITS (if any)
Laboratory of Neuropsychology, NIMH

LAB/BRANCH
Laboratory of Cerebral Metabolism, NIMH

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.25	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The deoxyglucose method is being applied to the monkey to advance knowledge regarding the parts of the brain which are involved in the processing of visual information. By measuring rates of local cerebral glucose utilization in animals during their performance of tasks involving different types of visual stimuli we anticipate learning which parts of brain are involved in such functions as discrimination, memory, and motivation. Also by studying animals at various ages, information will be obtained regarding the maturation of the visual processing system.

PHS-6040
(Rev. 10-76)

Project Description:

The goal of the collaborative effort initiated in 1978 was to map regions of monkey brain which were responsive to visual stimulation. The deoxyglucose method has been shown to be sensitive to even small differences in functional activity, and it was hoped that it might be possible to shed light on such complex aspects of visual function as discrimination, memory, or even the mechanism by which the brain assigns a value judgement on the character of visual stimulation and then initiates a response to it. The procedure followed is to prepare animals so that one hemisphere is completely deprived of visual input. One optic tract is sectioned as is the corpus callosum and forebrain commissures. Because the intact brain functions symmetrically and therefore has equal metabolic rates in all homologous structures, the finding of right-left differences in metabolic rates in the surgically prepared animals serves to identify the visually responsive regions. The experiments to date have demonstrated that these include the striate cortex and entire expanse of the circumstriate and inferior temporal cortex as far forward as the temporal pole.

A major project has been the delineation of the exact border between visual and non-visual cortex throughout this extended region. This has been facilitated by the computer-assisted image-processing system which makes possible the estimation of average values for histologically distinct cortical areas. The border separating visual from non-visual cortex has now been mapped in detail through the entire extent of striate cortex (Areas OB and OA) to the inferior convexity of the temporal lobe (Areas TEO and TE).

In other experiments the contribution of the commissural systems to these visually responsive cortical areas was determined. The commissural systems are those which transmit visual information from cortex across the mid-line to contralateral cortex. This was done by comparing average rates of glucose utilization in cortex in monkeys which had the optic tract alone sectioned with those which had had optic tract section plus commissural section. The results indicated that the commissural contribution is very largely due to a region designated TE in the anterior portion of the inferior temporal lobe.

In the experiments cited above many animals had been trained to respond to a specific visual stimulus with unimanual key-pressing to obtain a water reward. Thus the same experiments which were used to map the extended visual system also provided information on the metabolic responses to motor activity. While the motor pathways of the brain have been identified by other techniques, and thus are known, these experiments served to delineate the specific subdivisions of many structures which selectively are activated in the unimanual key-pressing. They provided new information on somatotopic localization of arm-hand movements. This was particularly well-defined in the study of cerebellar cortex. A large part of Crus II of the ipsilateral cerebellar cortex was shown to be selectively responsive in the animals' performance of the task. Also participating, but with a lesser percent change, was the lateral portion of the vermis in lobules III-VI. Localized increments in the rate of glucose utilization were also noted in VL and VPL of the thalamus, part of the globus pallidus and discrete zones of cerebral cortex (S, S_{II}, M) and a part of the supplementary motor area. A noteworthy feature of this

mapping study of motor activity is that a much greater metabolic increment was found in structures concerned with sensory monitoring of motor activity than in those related to the motor activity itself.

In progress is the analysis of the experimental results in animals studied during early development.

Yet to be undertaken are experiments which will compare the effects of different types of visual stimulation as well as visual stimuli in tasks which involve memory.

The Laboratory of Neuropsychology reports on this project with number Z01 MH 02033-04 LN, titled Functional Mapping of Sensory Systems.

Publications:

Kennedy, C., Miyaoka, M., Suda, S., Macko, K., Jarvis, C., Mishkin, M., and Sokoloff, L.: Local metabolic responses in brain accompanying motor activity. Trans. Amer. Neurol. Assoc. (in press) 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00889-02 LCM

PERIOD COVERED
October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

A Method for the Determination of Local Rates of Protein Synthesis in Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Smith	Staff Fellow	LCM NIMH
OTHER:	L. Sokoloff	Chief, Lab. of Cerebral Metabolism	LCM NIMH
	C. Kennedy	Guest Worker	LCM NIMH
	C. S. Patlak	Chief, Theoretical Statistics & Math.Br.	B NIMH
	K. Pettigrew	Mathem./Stat.,Theor.Stat.& Math.Br.	B NIMH
	S. Suda	Visiting Fellow	LCM NIMH
	F. Orzi	Visiting Fellow	LCM NIMH
	M. Ito	Visiting Associate	LCM NIMH
	J. C. Gillin	Chief, Unit on Sleep Studies	APB NIMH
	W. Mendelson	Research Psychiatrist	APB NIMH
	F. I. Storch	Psychology Technician	APB NIMH
	R. K. Nakamura	Senior Staff Fellow	LPP NIMH
	M. Mishkin	Acting Chief, Lab.of Neuropsychology	LN NIMH

COOPERATING UNITS (if any)

B. Agranoff, University of Michigan, Ann Arbor, Michigan
R. Collins, Washington University, St. Louis Mo.
M. Phelps, UCLA, Los Angeles, Calif.

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A method is being developed for the estimation of local rates of protein synthesis in brain in vivo. The method is based on the use of L-[1-^{14}C]leucine as a tracer for the incorporation of leucine into protein. A model of the behavior of leucine and [1-^{14}C]leucine has been designed. By mathematical analysis of the kinetics of exchange of the amino acid between plasma and tissue and its incorporation into protein, an equation has been derived that defines the rate of amino acid incorporation into protein in terms of the time course of plasma specific activity, final tissue concentration of ^{14}C, and experimentally determined kinetic constants. Tissue concentrations of ^{14}C are determined by quantitative autoradiography.

The method is currently being applied to studies of development, cretinism, plasticity in the visual system, seizures, regeneration, and sleep.

PHS-6040
(Rev. 10-76)

Project Description:

A method is being developed for the estimation of local rates of protein synthesis in brain in vivo. This method is similar to the [^{14}C]deoxyglucose method in that it is based on the principles of enzymology as applied to the measurement of reaction rates in vivo with labeled tracers as substrates. In order to measure the rate of the reaction, one must know the amount of product formed in a given interval of time and the integrated specific activity of the precursor. In an in vivo experiment the precursor pool cannot be sampled and the specific activity determined directly. It is necessary, therefore, to design a model for the behavior of the precursor in vivo and by kinetic analysis of the model to derive a relationship between the entire history of the precursor specific activity in the plasma (which can be sampled and measured directly), the integrated specific activity of the precursor pool in the tissue, and the rate of the reaction.

We have chosen L-[1-^{14}C]leucine as the radiolabeled tracer for this method because the $^{14}CO_2$ derived from its metabolism is rapidly diluted in the pool of CO_2 and cleared from the tissue. There are, therefore, no side-reactions with radioactive products other than the labeled protein. In our current model for the behavior of leucine and [1-^{14}C]leucine in the tissue, there are two pools of free amino acid in the tissue, the precursor pool for protein synthesis and the acid soluble pool of free amino acid. These two pools are connected in series with the plasma. The precursor pool rapidly equilibrates with the plasma and feeds into the "acid soluble" pool which turns over more slowly. We assume that there is no reutilization of either the labeled or unlabeled leucine derived from protein breakdown. The model requires a steady state for protein and amino acid metabolism. By mathematical analysis of the kinetics of the exchange of the amino acid between plasma and tissue and its incorporation into protein, an equation has been derived that defines the rate of leucine incorporation into protein in terms of the following measurable variables: the time course of plasma-specific activity, final tissue concentration of ^{14}C and experimentally determined kinetic rate constants.

The equation defines the procedure to be used and the variables to be measured. A pulse of [^{14}C]leucine (100 μCi/kg body weight) is administered intravenously, and the arterial plasma concentrations of labeled and unlabeled leucine are monitored for the duration of the experimental period. At 60 minutes the animal is killed by an intravenous injection of pentothal. The brain is removed, frozen and sectioned and local tissue concentrations of ^{14}C are determined by quantitative autoradiography.

The validity of the assumption that there is no reutilization of the unlabeled leucine from protein breakdown during the period of measurement remains to be proven. In vitro studies (Robertson and Wheatley, 1979) have shown that the precursor pool for protein synthesis is drawn directly from the extracellular space. Degradation of protein takes place within the cell, probably within lysosomes. This compartmentalization would preclude mixing of the leucine derived from protein degradation with the precursor amino acid pool for protein synthesis. In vivo, however, because the extracellular space is small, this mixing might occur outside the cell.

We are currently carrying out experiments to test this hypothesis. In these experiments we generate a square wave of [^{14}C]leucine in the arterial plasma of a rat and determine the specific activities of the leucyl-tRNA and the plasma leucine. If there is no reutilization from protein breakdown the leucyl-tRNA should reach the specific activity of the leucine in the plasma. If there is reutilization, it will never reach the specific activity of the plasma as it will be constantly diluted by unlabeled leucine from protein degradation. The rate at which the leucyl-tRNA reaches equilibrium is a function of the half-life of the precursor pool. These experiments require the isolation and the determination of picomolar levels of leucyl-tRNA. Methods have been developed for the extraction (with acetate, pH 4.0, buffered phenol) of aminoacyl-tRNA from brain, its deacylation at pH 10, and the determination of the specific activity of the leucine by a combination of dansylation with [^{3}H]dansylchloride, thin layer chromatography and double label, liquid scintillation counting.

We have determined rates of protein synthesis in a number of brain regions. We find a wide range of values from 1.5 nmoles leucine/g of tissue/min in white matter to 20 nmoles/g/min in some hypothalamic nuclei (e.g., the supraoptic nuclei). In general, brain regions that are rich in nerve cell bodies, such as the pyramidal cell layer in the hippocampus, and nerve nuclei such as the dorsal motor nucleus of the vagus, have high rates of protein synthesis as compared to either white matter or regions composed largely of nerve terminals, dendrites, synapses, and axons, such as the caudate nucleus, thalamus and cortex. The value that we have obtained for cortex (5.0 nmoles leucine/g/min) compares favorably with values obtained by Dunlop et al. (1975) of 4.7 nmoles valine/g/min with a completely different method.

We have embarked on several studies of the effects of specific treatments or conditions on local rates of protein synthesis. The purpose of some of these studies is to test the sensitivity of the method to detect changes in local rates of protein synthesis. Experiments conducted by Dr. R. Collins with our method have shown that bicuculline-induced seizures produce a reduction in protein synthesis. Studies of the effect of injury to the hypoglossal nerve have been carried out in collaboration with Dr. B. Agranoff. These studies have shown that cutting the hypoglossal nerve on one side will result in an increase in protein synthesis in its nucleus. We have studied the time course of this effect. We have also examined the time course of the effect of nerve section on glucose utilization in the nucleus. Our results show that the earliest change occurs in the rate of glucose utilization by 24 hours after nerve section. The increase in protein synthesis occurs later on day 4. The magnitude of the effect on glucose utilization is larger (70-80% increase over control) than that on protein synthesis (20-30%). Both of these metabolic responses return to normal by day 35. A functional connection between the nerve and tongue is restored by day 24. Other studies in progress in this laboratory include studies of the local rates of protein synthesis in brain during development, in cretinism, and during slow wave sleep.

The method is also being used to study the development and plasticity of the visual system in the newborn monkey. Prolonged monocular deprivation at birth results in a broadening of the ocular dominance columns representing the intact eye at the expense of the columns of the deprived eye. Eventually most of the

striate cortex may be incorporated into a monocular visual system serving only the undeprived eye. The process underlying this reorganization is unclear. There may be an accelerated growth of terminals from the functional columns into the adjacent columns or a reduced growth and consequent retraction of the terminals of the non-functional pathway or a combination of both. The perikarya of the terminals in the striate cortex are located in well-defined layers segregated for the right and left eyes in the lateral geniculate bodies. We applied the [^{14}C]leucine method to the study of protein synthesis in chronic and acute monocular visual deprivation in newborn monkeys. Chronic monocular deprivation resulted in decreased rates of protein synthesis in the laminae of the lateral geniculate nuclei innervated by the deprived eye whereas in geniculate laminae innervated by the functioning eye rates of protein synthesis were normal. Acute monocular deprivation produced no differential changes in rates of protein synthesis in any of the geniculate laminae. These results suggest that the underdevelopment of the deprived columns is the result of inadequate growth and/or maintenance of axon terminals with consequent default of synaptic connections to the normally maintained terminals of the functional pathway.

Proposed Course:

Plans are now being formulated in collaboration with Dr. Michael Phelps of the Division of Nuclear Medicine, UCLA, an organization that has a functional positron-emission tomographic laboratory, to adapt the method for use in man. In the Laboratory of Cerebral Metabolism, the method will be used to carry out or complete studies on thyroid hormone influences on brain development, plasticity of the visual system of primates, and slow-wave, and, perhaps, REM sleep, in the monkey.

Publications:

Kennedy, C., Suda, S., Smith, C.B., Miyaoka, M., Ito, M., and Sokoloff, L.: Changes in protein synthesis underlying functional plasticity in immature monkey visual system. Proc. Natl. Acad. Sci. USA 78: 3950-3953, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00900-25 LCM

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biochemical Studies on Myelin and Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Martenson	Research Chemist (Biochem.)	LCM	NIMH
Other:	G. E. Deibler	Chemist	LCM	NIMH
	M. L. Pedersen	Biologist	LCM	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three avenues of work are being pursued: (1) specific cleavages of the central nervous system myelin basic protein to obtain suitable fragments for biochemical and immunological studies, (2) identification of the specific amino acids in this protein that are phosphorylated in vivo, and (3) theoretical studies aimed at elucidating the secondary and tertiary structures of the myelin basic protein specific to the peripheral nervous system.

PHS-6040
(Rev. 10-76)

Project Description:

Our study on the isolation and identification of large fragments of rabbit myelin basic protein produced by limited peptic hydrolysis has been completed. All of the products expected from incomplete cleavages at the highly susceptible Phe^{44}-Phe^{45}, Phe^{87}-Phe^{88}, Leu^{109}-Ser^{110}, and Leu^{151}-Phe^{152} bonds have been isolated: peptides (1-151), (1-109), (1-87), (45-168), (45-151), (45-109), (88-168), (88-151), and (110-168). Of these, peptides (1-151), (1-87), and (88-151) were recovered in the greatest yield (0.14-0.19 mol/mol of starting protein). Lower yields (0.04 mol/mol of starting protein) were obtained for peptides (1-109) and (110-168), indicating that the Leu^{109}-Ser^{110} bond is somewhat more resistant to peptic cleavage than are the Phe-Phe and Leu-Phe bonds. Smaller fragments of the basic protein were also recovered in the study: peptides (1-44), (1-28), (45-87), (88-109), (110-151), and (152-168). It has been possible to identify many of the individual peptides in electrophoretograms of the total peptic digest. The relative mobilities of the above-mentioned peptides, together with peptides (1-14) and (15-44), have been determined in 15% (w/w) polyacrylamide slab gels containing 1 M acetic acid and 8 M urea.

Peptides (1-109), (45-168), and (45-151) are of particular interest in that they are missing one or both terminal sequences of the protein but contain the central region with intact Phe^{87}-Phe^{88} bond. The region surrounding this bond sequence 83-93, would appear to have a high potential for assuming an α-helical structure under favorable conditions; moreover, the integrity of this bond appears to be necessary, though not necessarily sufficient, for the basic protein to assume some α-helical structure when it binds to acidic lipid vesicles. The three peptides should be of considerable value in determining experimentally where the α-helix formation in the protein actually occurs under these conditions.

The study of the peptic peptides is being extended to the isolation of small fragments of the protein that contain phosphoserine and phosphothreonine residues. All of the previous studies on peptic peptides of the rabbit protein have involved the use of component 1, which is not phosphorylated. In the latest studies, we have started with a mixture of components 3, 4, and 5, which contains both phosphoserine and phosphothreonine. Initial fractionation of the peptic digests by gel filtration has shown that phosphoserine is present in two fractions, one of which is a mixture of peptides (1-44) and (45-87) and the other of which is a mixture of peptides (1-14) and (152-168). Phosphothreonine is present in the fraction consisting of peptides (15-44), (88-109), and (110-151). When the phosphorylated peptides are isolated by ion-exchange chromatography, they will be analyzed to determine which Ser or Thr residue in the sequence is phosphorylated. They will be used as standards in studies carried out by Dr. Harish Agrawal at Washington University to determine if these phosphate groups are the ones that are rapidly incorporated into the protein in vivo. Establishing the in vivo sites of phosphate turnover in the basic protein should help to define a little more clearly the role that this protein

plays in the myelin sheath.

The third avenue of work has involved a theoretical study of the structure of the P2 myelin protein. The P2 protein of peripheral myelin induces allergic neuritis in experimental animals. It is a relatively small, compact macromolecule whose secondary structure in aqueous solution, as revealed by circular dichroism, consists of about 70% β-structure. According to statistical and information theory predictive algorithms, many regions of the polypeptide chain have a high potential for forming either β-strands or α-helices, while other regions have a high potential for forming α-helices, but not β-strands, and vice versa. In the absence of physicochemical data to the contrary, the protein could be predicted as predominantly helical. An analysis of the amino acid sequence shows that the ten segments of the protein with β-structure potential, containing about 60% of the total residues, have their amino acids ordered such that one side of the β-strand is relatively hydrophobic, while the other side is relatively hydrophilic. It is concluded that this property, the separation of hydrophobic and hydrophilic surfaces, is responsible for providing the long-range interactions that lead specifically to extensive β-sheet, rather than α-helix, formation when the protein is in aqueous solution. On the basis of the secondary structure prediction and the orientation of the hydrophobic and hydrophilic surfaces of the strands, the tertiary structure would consist of two domains, each made up of five β-strands organized into two antiparallel sheets; the two domains would be joined by the formation of a parallel β-sheet. Upon binding to amphipathic molecules, considerable α-helix formation occurs, and a fairly extensive reorganization of the tertiary structure is predicted to result.

Publications:

Martenson, R.E., Lüthy, V., and Deibler, G.E.: Cleavage of rabbit myelin basic protein by pepsin. J. Neurochem. 36, 58-68, 1981.

Martenson, R.E.: Prediction of the secondary structure of myelin basic protein. J. Neurochem. 36, 1543-1560, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00901-26 LCM

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Immunologic Reactivity of Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH
Other:	J. R. Richert	Guest Worker	LCM	NIMH
	T. Namikawa	Visiting Fellow	LCM	NIMH

COOPERATING UNITS (if any)

National Multiple Sclerosis Society
E.E. Alvord, Jr., University of Washington School of Medicine, Seattle, Wash.

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Transfer of EAE with BP-sensitized Lewis rat spleen and lymph node cells has been used to study immune mechanisms in the induction and suppression of EAE. Specifically, we have attempted to define the conditions under which culture with specific antigen activates BP-sensitized spleen and LN cells as well as those conditions which might generate antigen-specific suppressor cells in culture.

PHS-6040
(Rev. 10-76)

Project Description:

In studying mechanisms of activation and suppression of EAE lymphoid cells in the Lewis rat, we previously reported that both spleen cells and lymph node cells (LNC) from myelin basic protein (BP)-sensitized rats can be activated by culture with BP. Activated cells are capable of transferring disease 100-1,000 times more efficiently than cells that have not been cultured with antigen. Cellular proliferation, measured by tritiated thymidine (^{3}H-TdR) uptake is also enhanced by addition of BP to cultured cells. Those studies were carried out with cells from donors sensitized with BP/CFA. If these donors had not been sacrificed for cell transfer, 100% of them would have become paralyzed as a result of BP/CFA sensitization.

Dr. Tadashi Namikawa has continued Dr. Richert's study of mechanisms involved in EAE induction and suppression. EAE (induced as above) can be prevented by multiple injections of BP in incomplete Freund's adjuvant. The difference between the two immunization techniques is the presence of mycobacteria in the inoculum. BP without mycobacteria will not induce disease in rats but instead elicits a resistant state. It has been claimed that spleens or lymph nodes from animals protected in this manner contain suppressor cells because cell transfer protected the recipient from subsequent challenge. We incubated Sp C from protected animals with antigen to see if suppression could be enhanced. Instead we found that after in vitro exposure to antigen they transferred EAE as well as BP/CFA Sp C.

This result was totally unexpected in view of the report that BP/IFA sensitization induced suppressor cells. It should be pointed out that in order to achieve suppression large numbers of these cells were transferred directly without any exposure to antigen. We do not know whether culture elicits effector cells at the expense of the suppressors or whether the phenomenon of suppression was caused by some mechanism other than suppressor cells per se.

There is another difference between these two populations: In addition to the fact that BP/IFA cells do not proliferate in response to BP (while BP/CFA cells do) BP/IFA cells fail to transfer EAE after incubation with Con A as BP/CFA cells do. (Con A is a T cell mitogen which markedly enhances the proliferative response of naive as well as sensitized T cells. These reactions are summarized in a Table on the next page.

The current study shows that some mechanism other than the simple induction of suppressor T-cells is probably responsible for the unreactive state induced by hyperimmunization of rats with BP in IFA. Whatever control is responsible for preventing BP/IFA cells from expressing their potential for CNS damage, it is abrogated by exposure of these cells to specific antigen in vitro.

Different Behavior of BP/CFA and BP/IFA Sensitized Cells

Incubation with:	BP/CFA (encephalitogenic in donor)	BP/IFA (non-encephalitogenic in donor)
Antigen (BP)	Strong proliferative response. Enhanced transfer	No proliferative response. Enhanced transfer
Mitogen (Con A)	Strong proliferative response. Enhanced transfer	Strong proliferative response. No enhanced transfer

Publications:

Richert, J. R., Driscoll, B. F., Kies, M. W., and Alvord, E. C., Jr.: Experimental allergic encephalomyelitis: activation of myelin basic protein-sensitized spleen cells by specific antigen in culture. Cell. Immunol. 59: 42-53, 1981.

Richert, J. R., Kies, M. W., and Alvord, E. C., Jr.: Enhanced transfer of experimental allergic encephalomyelitis with Lewis rat lymph node cells. J. Neuroimmunol. 1: 195-203, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00902-16 LCM

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Induction and Prevention of Experimental Allergic Encephalomyelitis (EAE)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. F. Driscoll	Guest Worker	LCM	NIMH
Other:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH

COOPERATING UNITS (if any)
National Multiple Sclerosis Society
E. C. Alvord, Jr. University of Washington School of Medicine, Seattle, Wash.

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In attempting to dissect the various cellular events responsible for the development of EAE, we have studied the effect of in vitro culture with antigen on the ability of BP-sensitized cells to transfer EAE to naive recipients. Two different cell compartments, peritoneal exudate cells, PEC, and lymph node cells, LNC, display distinctly different responses to BP in culture. In vivo, these two cell populations differ in the expression of cell surface markers (Ia antigens). PEC are Ia+ and LNC are Ia-. In contrast, after culture for 24 hours both populations are Ia+. They both appear to be activated in vitro by BP and fragments of BP which contain the GP encephalitogenic site.

PHS-6040
(Rev. 10-76)

Project Description:

EAE is induced in susceptible animals by injection of purified myelin basic protein (BP) in complete Freund's adjuvant (CFA). The subsequent immune response leading to the development of disease is T cell mediated and assumed to be delayed type hypersensitivity reaction (DTH). Animals develop positive skin reactions when injected with BP and their cells proliferate and produce lymphokines upon contact with BP in vitro.

We have reported that very low numbers of peritoneal exudate cells (PEC) from BP/CFA sensitized guinea pigs can transfer severe EAE after culture with antigen (BP). Also, lymph node cells (LNC) transfer EAE in an enhanced fashion after culture with antigen if a second non-specific stimulus is provided by the presence in culture of allogeneic (Strain 2) PEC.

It is quite clear that immune responsiveness requires not only antigen recognition but also communication between various stimulatory or inhibitory populations of cells. Communication between these cells depends on their sharing certain cell surface antigens (glycoproteins) which are encoded by the major histocompatibility gene complex (MHC). For instance, antigen-presenting cells must share certain cell surface markers with T cells in order to present antigen efficiently to immune T cells. Of particular importance to EAE is the observation that successful transfer of DTH between various inbred strains of mice required that the cell donor and recipient must share at least some genes in the immune (I region) of the MHC. Thus, the I region of the MHC and the Ia (I region associated) cell surface antigens which are encoded there play a central role in the control of DTH responses.

Strain 13 and Strain 2 guinea pigs share identical MHC genes with the exception of the I region. While the two strains express some shared Ia antigens, the Strain 13 cells have Ia-1,3,7 antigens not found on Strain 2 cells and Strain 2 cells have Ia-2,4 antigens not found on Strain 13 cells. Taking advantage of these known differences, we were able to induce anti-Ia-1,3,7 antiserum (in Strain 2 animals) and anti-Ia-2,4 antiserum (in Strain 13 animals) by immunization of either strain with lymphoid cells of the other strain. We then used these antisera to ascertain the role of Ia+ cells in EAE, and in particular, the role of such cells in enhanced transfer.

Several interesting observations resulted from these experiments. First, the Strain 2 PEC used as a non-specific stimulus in LNC cultures are Ia+ cells. Treatment of these cells with anti-Ia-2,4 and complement before culture inhibits their ability to induce a mixed lymphocyte reaction and this, in turn, inhibits the ability of the LNC to transfer EAE after culture.

Using anti-Ia-1,3,7, we found that treatment of BP-sensitized Strain 13 PEC either before or after culture depresses their ability to transfer EAE;

this means that the EAE effector cells in the PEC are predominantly Ia+ both in the animal (i.e. before culture) and after culture. In contrast, treatment of BP-sensitized LNC before culture had no effect on disease transfer but treatment of such cells after culture severely inhibited transfer of EAE. The LNC are clearly Ia- in the animal and Ia+ after 24 hr culture. However, the significance of the results obtained after culture is diminished by the fact that transfer of EAE by the Strain 13 LNC after culture is also inhibited by anti-Ia-2,4 antiserum. This means that the Strain 13 cells are passively acquiring Strain 2 alloantigens during the 24 hours of culture and might also be passively acquiring the Strain 13 alloantigens. The typing of the cells for the presence of Ia antigens after culture might lead to an incorrect assessment of the cells phenotype. It is clear, however, that BP-sensitized cells found in different compartments of the animals are expressing different amounts of Ia antigen - LNC clearly have little Ia antigen on their surface and PEC do have Ia antigen on their surface. These studies should prove useful in probing the role of various cell subpopulations in the disease process and might be useful in isolating particular populations for further study.

A second area of research has been an investigation of the role that antigen plays in culture. These experiments were aimed at defining the molecular requirements the antigen must possess to enhance transfer. BP was replaced in culture by well characterized proteolytic fragments of the whole molecule. The results indicate that only fragments containing the GP encephalitogenic site (Res. 113-121) can activate cells for enhanced transfer. Non-encephalitogenic fragments induce neither proliferation in BP/CFA sensitized PEC nor enhanced transfer by such cells. During these studies we discovered that our preparation of the N-terminal portion of BP (Res. 1-88) which does not contain the GP encephalitogenic site, is probably contaminated with a very small amount of an encephalitogenic fragment which is located in the C-terminal portion of BP. To date, the contaminant has been detected only by this sensitive cell test. All biochemical procedures have failed either to detect the presence of the contaminant or to separate it physically from the major component (Res. 1-88). Chemical studies have been described in Mrs. Deibler's report.

Publications:

Driscoll, B. F., Kies, M. W., and Alvord, E. C., Jr.: Enhanced transfer of experimental allergic encephalomyelitis with strain 13 guinea pig lymph node cells: requirement for culture with specific antigen and allogeneic peritoneal exudate cells. J. Immunol. 125: 1817-1822, 1980.

Seil, F. J., Kies, M. W., and Bacon, M. L.: A comparison of demyelinating and myelination-inhibiting factor induction by whole peripheral nerve tissue and P_2 protein. Brain Res. 210: 441-448, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00903-04 LCM

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Preparation and Identification of Peptides Derived From Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Myelin basic protein (BP) presents a unique opportunity for a detailed study of the relationship between structure and biological activity--it has been completely sequenced and there is an impressive backlog of information on its encephalitogenic activity in several species; its ability to induce and bind antibody and its ability to bind other biochemical compounds or structures, specifically membrane constituents.

In order to study the relationship between structure and activity, we have prepared several well-characterized fragments of the protein by controlled proteolytic digestion. Our purpose is to test the ability of these peptides to replicate the biological activity of the protein. By using fragments from different regions of the protein one can "map" active sites and define the minimal structural requirements for activity.

PHS-6040
(Rev. 10-76)

Project Description:

BP-fragments prepared by limited peptic digestion and purified by ion-exchange chromatography at pH 8.0 appeared to be homogeneous. Fragment 89-169, which contains the guinea pig encephalitogenic site (Res. 113-122) stimulates cellular proliferation and enhances transfer of EAE with BP-sensitized cells and is also capable of inducing such cells in vivo. In contrast, sensitization with fragment 1-88 does not induce cells capable of proliferation or enhanced transfer; nevertheless, 1-88 has stimulated and enhanced BP-sensitized cells in vitro. Except for these experimental results, our data had shown that the encephalitogenic site (Res. 113-122 -- Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys) was necessary for any stimulation of proliferation or enhanced transfer of EAE. Electrophoretic analysis of 1-88 showed only minor amounts of impurities and amino acid analysis yielded a good composition. However, the C-terminal analysis yielded a small amount of Ala and Arg, indicating a slight amount of contamination with some portion of the carboxyl half of BP. This contaminant might account for the unexpected activity in Fragment 1-88.

Further purification of 1-88 by gel filtration, which was monitored at 225 nm (absorption of peptide bond), 275 nm (absorption of tyrosine) and 290 nm (absorption of tryptophan), yielded some lower molecular weight material. The ratio of 290/275 indicated the presence of tryptophan in the trailing edge of the main peak and also in the lower molecular weight fraction. This was an important observation because the encephalitogenic site contains the only tryptophan residue in the BP molecule. The major peak was subdivided into 5 fractions. Polyacrylamide slab gels showed that the first and last fractions were clearly contaminated and that the smaller molecular weight material contained one major band and 5 minor bands. The major band of the smaller molecular material was tentatively identified as 111-169 by amino acid analysis and C-terminal analysis. The 3 center fractions of the main peak had the amino acid composition of 1-88. However, these fractions which appeared to be homogeneous, as well as the fractions which clearly were contaminated, still retained in vitro activity.

The major peak from the gel filtration experiment was subjected to ion-exchange chromatography at pH 4.5, yielding one major peak and a minor peak. The minor peak was collected separately and the major peak was cut into 3 fractions. Each fraction was then purified by gel filtration. The center fraction again yielded in vitro activity. This activity is either due to a site in 1-88 which cross-reacts with the encephalitogenic site or to trace contamination with an encephalitogenic peptide similar to 1-88 in both molecular weight and charge. In either case, in vitro activation of BP-sensitized cells is a much more sensitive means of detecting either intrinsic biological activity or impurity than is bioassay or gel electrophoresis. Further purification is being carried out by high pressure liquid chromatography and a new ultra-sensitive electrophoretic technique.

Publications:

DeArmond, S., Deibler, G. E., Bacon, M. L., Kies, M. W., and Eng, L. F.: A correlated neurochemical and immunocytochemical study of P2 protein in the human and bovine nervous systems. J. Histochem. Cytochem. 28: 1275-1285, 1980.

Sires, L. R., Hruby, S., Alvord, E. C., Jr., Hellstrom, I., Hellstrom, K. E., Kies, M. W., Martenson, R., Deibler, G. E., Beckman, E. C., and Casnellie, J. E.: Species restrictions of a monoclonal antibody reacting with residues 130-137 in encephalitogenic myelin basic protein. Science. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00931-08 LGCB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies on the Characteristics and Regulation of S-Adenosylhomocysteine Hydrolase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Cantoni	Chief, Lab. Gen. Comp. Biochem.	LGCB NIMH
	P. K. Chiang	Research Biologist	LGCB NIMH
	R. R. Aksamit	Senior Staff Fellow	LGCB NIMH
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LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2-5/6	5/6	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A large number of chemicals that are either analogs of adenosine or of adenosylhomocysteine have been examined for their ability to function as inhibitors and/or substrates of adenosylhomocysteinase.

The two most interesting compounds studied are 3-deazaadenosine and 3-deazaaristeromycin. Both are inhibitors of adenosylhomocysteinase but the nature of their inhibitory properties is different in that 3-deazaaristeromycin unlike 3-deazaadenosine is an irreversible or suicidal inhibitor of the enzyme.

In vivo both compounds affect the intracellular levels of S-adenosylmethionine, and adenosylribosyl homocysteine, and inhibit a variety of methylation reactions. Moreover, they exhibit a variety of interesting biological activities such as antiviral activity against a number of RNA and DNA viruses, and are able under certain conditions to prevent cellular transformation induced by pncopgeni viruses. 3-Deazaadenosine, but not 3-deazaaristeromycin inhibits chemotaxis by a mouse macrophage cell line.

This interesting difference may provide the opportunity to elucidate the biochemical reactions of importance in eukaryote chemotaxis, and can be used as a model for the search and development of other specific inhibitors of transmethylation reactions.

Project Description:

As is well known S-adenosylmethionine (AdoMet) is a key intermediate in biological transmethylation and transalkylation reactions. There are hundreds of reactions, each catalyzed by a specific enzyme, that utilize AdoMet as a substrate. It is obvious, therefore, that the utilization of AdoMet in biological systems must be under regulatory controls but, at the present time, little is known about the nature of these regulatory control mechanisms.

While the biochemical mechanisms of transmethylation reactions have been elucidated many years ago, largely as a result of the studies by Cantoni and his collaborators at NIH, the correlation between many methylation reactions and cellular functions remains obscure. Thus, for instance, the significance of the methylation of a variety of informational macromolecules such as proteins and nucleic acids (DNA, ribosomal-, messenger-, viral and tRNA, etc.), or of complex polysaccharides, or even simpler compounds such as guanido acetic acid, nicotinamide, etc. is not immediately obvious and is the subject of much debate.

It has been established, however, that S-adenosylhomocysteine (AdoHcy), one of the products of transmethylation reactions that utilize AdoMet as a methyl donor is a competitive inhibitor of many, if not most, of the reactions in which AdoMet participates. As the result of work in this and other laboratories, it has been proposed as a working hypothesis that the intracellular ratio of AdoMet/AdoHcy must be of key importance in the regulation of biological alkylation reactions and play a role in the determination of the hierarchy of biological methylation or, more generally, biological alkylation reactions. It can also be surmised that modulation of AdoMet/AdoHcy ratio might result in important, and perhaps unexpected physiological effects, that, if correlated with biochemical data, might give a clue as to the significance of different methylation reactions.

In bacteria, use of mutants is of great help in the analysis of problems of this type. In eukaryotes, and especially vertebrates, other approaches had to be devised. It is a fortunate fact that in eukaryotes AdoHcy is metabolized through a single metabolic pathway by S-adenosylhomocysteinase (AdoHcyase), an enzyme that catalyzed the reversible hydrolysis of AdoHcy to adenosine (Ado) and homocysteine (Hcy). We decided some years ago to take advantage of this fact and initiated a long range experimental project designed first to study in depth the properties of AdoHcyase and then to develop a series of specific inhibitors of this enzyme. As the result of our own studies and studies in other laboratories on the properties and kinetic characteristics of AdoHcyase, we have now established the fact that by use of specific inhibitors of the enzyme it is possible to control the intracellular levels of AdoHcy and/or to accumulate intracellularly congeners of AdoHcy, of the general formula S-purinylhomocysteine (PurHcy). By use of these inhibitors it is now possible to modulate in different cellular systems the AdoMet/AdoHcy and/or AdoMet/PurHcy ratio and to examine the consequences of these changes.

Our studies confirmed and extended in other laboratories have shown that inhibitors of AdoHcyase may be divided into two groups: a) competitive inhibitors that can also be utilized as substrates by the enzyme, and b) irreversible or suicidal inhibitors that bind to the active center of the enzyme and inactivate it irreversibly.

The prototype of the first group of inhibitors is 3-DZA, an analogue of adenosine extensively studied in this laboratory. We have shown that in vitro 3-deazaadenosine (3-DZA) is a very potent competitive inhibitor of AdoHcyase with a K_i of 5-8 μM; as a substrate it is about equivalent to the natural substrate, adenosine with a similar affinity for the enzyme.

In vivo administration of 3-DZA to laboratory animals or to a variety of cellular systems results in the intracellular accumulation of: a) AdoHcy, as a result of the partial inhibition of AdoHcyase; and b) 3-DZA-Hcy as a consequence of the fact that whatever homocysteine is released from AdoHcy by the action of the partially inhibited AdoHcyase can be utilized by the enzyme for the synthesis of 3-DZA-Hcy. One of the important and unique features of 3-DZA is that unlike adenosine and other competitive inhibitors of AdoHcyase, 3-DZA is not a substrate for adenosine deaminase or adenosine kinase, two enzymes with ubiquitous cellular distribution. Under physiological conditions, these two enzymes metabolize adenosine very efficiently and displace the equilibrium of the reaction catalyzed as AdoHcyase in the direction of hydrolysis.

It would be expected that the intracellular accumulation of AdoHcy and/or 3-DZA-Hcy and the accompanying changes in AdoMet/AdoHcy ratio would result in inhibition of a number of transmethylases that would be reflected in an increase in the intracellular level of AdoMet (a consequence of its underutilization) and in a decrease in the intracellular concentration of many methylated intermediates. We have been able to verify experimentally this prediction and demonstrate a striking decrease in the amount of many methylated compounds, methylated phospholipids and creatine in the liver and carboxymethylated proteins in erythrocytes.

Another adenosine analog, 3-DZA aristeromycin synthesized in collaboration with Dr. J. Montgomery of the Southern Research Laboratories and first studied in this laboratory is a prototype of the second group in inhibitors: the so called suicidal or irreversible inhibitors. Unlike the compounds of group A that act as inhibitors and/or substrates, compounds in this group, when administered in vivo bring about only accumulation of AdoHcy.

Accumulation of AdoHcy and/or 3-DZA-Hcy is accompanied in a variety of different cellular synthesis by striking biological effect such as resistance to chemotactic and phagocytic stimuli, to infection by various RNA and DNA viruses, etc.

Studies in this and other laboratories of a large number of synthetic analogs of AdoHcy as inhibitors of reactions involving transfer of the methyl group of AdoMet to a variety of substrates has revealed the interesting fact that the sensitivity of different transmethylases to AdoHcy and its analogs varies over a very wide range. Unfortunately, however, cellular membranes are relatively impermeable to AdoHcy and its analogs, so that it has not been possible to take advantage of the in vitro specificity of different analogs in in vivo experiments.

Comparison of the biological effects of 3-DZA and 3-DZA arysteromycin in a mouse macrophage cell line has yielded some very interesting and exciting results because it has been found that macrophage chemotaxis is specifically inhibited by the intracellular formation of 3-DZA-Hcy and not affected by accumulation of AdoHcy. This observation is of special interest from several points of view. In the first place it serves to confirm the validity of the hypothesis that has

guided our work on AdoHcyase and its role in biological methylation. It has now been demonstrated experimentally that it is possible to generate intracellularly a congener of AdoHcy with specific inhibitory characteristics. This observation suggests further that it should be possible to design other adenosine analogs capable of being utilized as substrates by AdoHcyase to generate intracellularly a family of target-directed inhibitors. In the second place, it should be pointed out that while it has been established in bacteria that protein carboxymethylation is essential for the chemotactic response, the biochemical requirements for eukaryotic chemotaxis are as yet not known. The discovery that macrophage chemotaxis can be specifically inhibited by 3-DZA-Hcy will open the way to elucidation of this important question. Chemotaxis is a reaction of particular importance not only in the immume response but also in the development of the central nervous system.

Publications:

Trager, W., Tershakovec, M., Chiang, P.K., and Cantoni, G.L.: Plasmodium falciparum in culture: antimalarial activity of sinefungin and other methylation inhibitors. Exp. Parasitol. 50: 83-89, 1980.

Phyall, W., Chiang, P.K., Cantoni, G.L., and Lovenberg, W.: The hypotensive action of 3-deazaadenosine. Eur. J. Pharmacol. 67: 485-488, 1980.

Morita, Y., Chiang, P.K., and Siraganian, R.P.: Effect of inhibitors of transmethylation on histamine release from human basophils. Biochem. Pharmacol. 30: 785-791, 1981.

Chiang, P.K.: Conversion of 3T3-L1 fibroblasts to fat cells by an inhibitor of methylation: effect of 3-deazaadenosine. Science 211: 1164-1166, 1981.

Bodner, A.J., Cantoni, G.L., and Chiang, P.K.: Anti-viral activity of 3-deazaadenosine and 5'-deoxy-5'-isobutylthio-3-deazaadenosine (3-deaza-SIBA). Biochem. Biophys. Res. Commun. 98: 476-481, 1981.

Johnson, G.S., and Chiang, P.K.: 1-Methylnicotinamide and NAD metabolism in normal and transformed rat kidney cells. Arch. Biochem. Biophys., in press.

Pritchard, P.H., Chiang, P.K., Cantoni, G.L., and Vance, D.E.: Inhibition of phosphatidylethanolamine N-methylation by 3-deazaadenosine stimulates the synthesis of phosphatidylcholine via the CDP-choline pathway. J. Biol. Chem., in press.

Aksamit, R.R., Falk, W., and Cantoni, G.L.: Inhibition of chemotaxis by S-3-deazaadenosylhomocysteine in a mouse macrophage cell line. J. Biol. Chem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00933-07 LGCB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Study of Adenosylmethionine Synthetase and Enzymes of Transmethylations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Cantoni	Chief, Lab. Gen. Comp. Biochem.	LGCB NIMH
	P. K. Chiang	Research Biologist	LGCB NIMH
Other:	I.-K. Kim	Visiting Fellow	LGCB NIMH
	T. M. Caryk	Chemist	LGCB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of General and Comparative Biochemistry

SECTION
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NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The molecular properties and kinetic characteristics of various enzymes involved in the metabolism of S-adenosylmethionine have been investigated. 5'-Methylthioadenosine nucleosidase from lupin seeds has been purified to homogeneity, and the molecular weight, subunit structure and kinetic characteristics of the enzyme has been investigated. The irreversible inhibition and substrate specifics of S-adenosylhomocysteinase from various sources have been investigated in considerable detail. S-Adenosylhomocysteinase from hamster liver has been purified to homogeneity and its kinetic characteristics, and sensitivity to inhibitors has been compared to that of the enzymes from beef liver, previously crystallized by Chiang, Richards and Cantoni.

Project Description:

In the reaction adenosine + L-homocysteine === S-adenosyl-L-homocysteine, catalyzed by S-adenosylhomocysteine hydrolase from beef liver (EC 3.3.1.1), eleven nucleosides are able to substitute for adenosine to generate their corresponding S-nucleosidylhomocysteine cogeners: 3-deazaadenosine, 2-aza-3-deazaadenosine, nebularine (purine ribonucleoside), formycin, N^6-methyladenosine, 8-azaadenosine, adenosine N^1-oxide, pyrazomycin, 8-aminoadenosine, inosine and the carbocyclic analogue of adenosine [(±)-aristeromycin]. S-Adenosylhomocysteine hydrolase from lupin seeds is able to utilize all of these nucleosides except inosine to synthesize analogues of S-adenosylhomocysteine. There is no correlation between the ability of these nucleotides to function as substrates and their inhibitory potencies, except in the case of 3-deazaadenosine. The carbocyclic analogue of adenosine is the most potent inhibitor of S-adenosylhomocysteine hydrolase with a K_i of 5×10^{-9} M.

Other adenosine analogs were found to inactivate AdoHcyase in a time dependent irreversible fashion. Among the more potent compounds are Ara-A and arysteromycin. The mechanism of catalysis described for AdoHcyase by Abeles and coworkers predicts transient formation of 3'-ketoadenosine. This intermediate, because of its chemical structure undergoes spontaneous decomposition with the liberation of adenine. Adenine liberation can therefore be used as an indicator of nature of the irreversible inhibition and may serve to differentiate various types of irreversible inhibitors.

The molecular and kinetic characteristics of AdoHcyase from different sources is being explored. As a part of this program, the enzyme from hamster liver has been purified and its interaction with 3-DZA and other inhibitors analyzed in great detail.

Alkyl transfer other than methyl transfer from AdoMet results in the formation of thiomethyladenosine. The metabolism of this intermediate is different in different phyla. As part of our interest in the metabolites of AdoMet, we have purified to homogeneity and characterized the enzyme from lupin seed responsible for the hydrolysis of thiomethyladenosine to adenine and thiomethylribose. The lupin seed enzyme differs from both the bacterial and the vertebrate enzymes that metabolize thiomethyladenosine. An evolutionary relationship between these enzymes is suggested by a comparison of their kinetic and molecular characteristic.

Publications:

Guranowski, A., Montgomery, J.A., Cantoni, G.L., and Chiang, P.K.: Adenosine analogs as substrates and inhibitors of S-adenosylhomocysteine hydrolase. Biochemistry 20: 110-115, 1981.

Chiang, P.K., Guranowski, A., and Segall, J.E.: Irreversible inhibition of S-adenosylhomocysteine hydrolase by nucleoside analogs. Arch. Biochem. Biophys. 207: 175-184, 1981.

Guranowski, A.B., Chiang, P.K., and Cantoni, G.L.: 5'-Methylthioadenosine nucleosidase: purification and characterization of the enzyme from Lupinus lacteus seeds. Eur. J. Biochem. 114: 293-299, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00934-09 LGCB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies on the Biochemical Basis of Narcotic Drug Action

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	L. Hjelmeland	Senior Staff Fellow	DPS NICHHD
	J. Hurwitz	Fogarty Scholar in Residence	NIH
	M. E. Ahmed	Guest Worker	LGCB NIMH

COOPERATING UNITS (if any)

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Section on Proteins

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Opiate receptors solubilized with the zwitterionic detergent, CHAPS, behave as macromolecular complexes carying at least two activities: reversible binding of opiate ligands, and a guanine nucleotide (and Na^+) sensitive regulatory activity. In membranes prepared from neuroblastoma x glioma hybrid cell NG108-15 or rat striatum, opiate receptors inhibit adenylate cyclase activity by stimulatory GTP hydrolysis catalyzed by a specific, low K_m, GTPase. These observations provide a general mechanism for the action of opiate receptors and for other inhibitory receptors coupled to adenylate cyclase.

Project Description:

Over the past several years we have been engaged in the characterization of the interaction between opiate receptors and adenylate cyclase in a cultured neuronal cell line, NG108-15. The cell has a large number of opiate receptors which function to inhibit adenylate cyclase and thereby lower cAMP levels. In analogy to the addictive process, the cells become tolerant to, and dependent upon, opiates after prolonged exposure. This adaptive response is due to a gradual increase in adenylate cyclase activity which serves to maintain normal cAMP levels in the continued presence of opiates. In the past year we have made significant progress in our understanding of the detailed mechanism of some of these opiate actions. Moreover, work in this and other laboratories has shown that the cyclic nucleotide linked mechanism of opiate action is operative in brain as well as in the cultured cell system.

As a first step towards the purification and reconstitution of the cellular constituents involved in opiate action, we have solubilized opiate receptors from several sources using the zwitterionic detergent, CHAPS. Receptors which reversibly bind opiate ligands with the appropriate specificity have now been isolated from membranes of NG108-15 cells, brain tissue (both rat and beef), and human placenta. Each of these receptor preparations behaves as a macromolecular complex of Stokes radius near 7 nm and contains protein as an essential constituent.

The guanine nucleotides GDP, GTP and guanosine-5'(β,γ-imido)triphosphate have been found to inhibit binding of opiates and opioid peptides to receptors solubilized from neuroblastoma x glioma NG108-15 hybrid cells. The inhibition requires sodium ions as well and reflects a decrease in affinity of receptors for opioid ligands. Whereas in membranes, only opioid agonist binding is decreased by guanine nucleotides, binding of both the agonist, etorphine, and the antagonist, deprenorphine, is reduced in the case of soluble receptors. These observations are consistent with the suggestion that solubilized receptors are complexes composed of an opiate binding protein and a guanine nucleotide regulatory component. Indeed, when such preparations are subjected to gel exclusion chromatography, opiate binding activity migrates together with the guanine nucleotide regulatory protein of adenylate cyclase.

The mechanism of opiate inhibition of adenylate cyclase has been unknown. Several clues to such a mechanism pointed towards the GTP regulatory system of the enzyme. These include a requirement for GTP (and Na^+) for opiate inhibition and the close association of receptors with the nucleotide regulatory protein already discussed. Because opiate inhibition of adenylate cyclase activity is blocked by hydrolysis-resistant GTP analogues it was likely that opiates act at the GTP hydrolysis step of the adenylate cyclase regulatory cycle. This hypothesis has now been substantiated by experiment. When tested under the appropriate conditions (which are also suitable for measurement of adenylate cyclase activity), GTP hydrolysis, catalyzed by a specific GTPase, is stimulated approximately two-fold by opiate agonists. Stimulation of GTPase is mediated by opiate receptors because: it is blocked by naloxone; requires the naturally (-)enantiomers of opiate agonists; the potencies of a series of opiates in this assay are well correlated with their affinities for the receptor. Importantly, opiate mediated stimulation of GTPase activity requires Na^+, just as does opiate inhibition of adenylate cyclase activity. Thus, opiate receptor mediated stimu-

lation of GTPase supplies a reasonable mechanism by which adenylate cyclase is inhibited. Other receptors, coupled to adenylate cyclase as inhibitors, such as α-adrenergic and muscarinic receptors may function by similar mechanisms.

Recent work performed independently in three different laboratories has clarified the conditions necessary to show inhibition of adenylate cyclase by opiates in rat brain membranes. We have confirmed some of these observations with membranes prepared from rat striatum. Inhibition of adenylate cyclase activity can now be considered a quite general mechanism of opiate action. Because of these observations we have begun to study opiate receptor mediated effects upon brain GTPase. Even though these studies are in their early steps, we can state with confidence that opiate receptor occupancy is coupled to stimulation of GTPase in brain just as it is in neuroblastoma x glioma cell hybrids. The detailed mechanism of action of opiates in brain membranes is therefore similar or identical to the mechanism as we currently understand it in cultured neural cells.

Publications:

Klee, W.A., and Streaty, R.A.: Multiple effects of opioid-receptor interactions in a clonal cell line. In Barker, J.L., and Smith, T.G., Jr. (Eds.): The Role of Peptides in Neuronal Function. New York, Marcel Dekker, 1980, pp. 712-726.

Simonds, W.F., Koski, G., Streaty, R.A., Hjelmeland, L.M., and Klee, W.A.: Solubilization of active opiate receptors. Proc. Natl. Acad. Sci. U. S. A. 77: 4623-4627, 1980.

Koski, G., Simonds, W.F., and Klee, W.A.: Guanine nucleotides inhibit binding of agonists and antagonists to soluble opiate receptors. J. Biol. Chem. 256: 1536-1538, 1981.

Ahmed, M.S., Byrne, W.L., and Klee, W.A.: Solubilization of opiate receptors from human placenta. Placenta, in press.

Koski, G., and Klee, W.A.: Opiates inhibit adenylate cyclase by stimulating GTP hydrolysis. Proc. Natl. Acad. Sci. U. S. A., in press.

Klee, W.A., and Streaty, R.A.: Opiate dependent dual regulation of adenylate cyclase activity in a cell-free system. Adv. Cyclic Nucleotide Res. 14: in press.

Klee, W.A., and Zioudrou, C.: The possible actions of peptides with opioid activity derived from pepsin hydrolysates of wheat gluten and of other constituents of gluten in the function of the central nervous system. In Hemmings, H. (Ed.): The Biochemistry of Schizophrenia and Addiction. Great Britain, MTP Press, 1980, pp. 53-76.

Rapoport, S.I., Klee, W.A., Pettigrew, K.D., and Ohno, K.: Entry of opioid peptides into the central nervous system. Science 207: 84-86, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00935-14 LGCB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effect of Small Viruses and Their Nucleic Acids on the Biochemistry of Living Organisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. R. Merril	Senior Research Scientist	LGCB	NIMH
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	S. L. Adhya	Geneticist	LMB	NCI
	J. Horst	Professor, Human Genetics, University of Ulm, West Germany		
	J. Leavitt	Biochemist, Division of Virology		FDA
	W. G. Nash	Expert Consultant	LVC	NCI

COOPERATING UNITS (if any)

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Division of Virology, Bureau of Biologics, FDA
Dept. of Human Genetics, University of Ulm, West Germany

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section of Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1	2

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Expression of the E. coli gal operon under the control of the prophage lambda promoter, p_L leads to gross discoordinancy of gal expression. A survey of protein synthesized after prophage induction indicated that lack of expression of galE is due to a failure of translation of galE. Failure of translation of the galE sequence may be due to extensive dyad symmetry present in or near the gal promoter. This model was tested with insertions, deletions, or new promoter mutations within the symmetrical region. These strains restored some galE expression indicating that the RNA stem-loop structure plays a role in the discoordinate expression of gal from p_L.

Co-infection of mammalian cells with the E. coli gal-kinase operon and the herpes virus thymidine kinase genes has been used under selective conditions (for thymidine kinase) to obtain clones of cells which are transcribing and translating the E. coli gal-kinase gene.

Drosophila melanogaster was surveyed for the enzymes of the Leloir pathway, no enzyme activity was detected, with the exception of trace amounts of 5-uridine diphosphogalactose-4-epimerase activity in the egg. This animal may be of use to study in vivo expression of the E. coli galactose genes.

Project Description:

Bacterial Genes in Bacteria - We have extended our observation that the expression of a bacterial cistron can depend upon the promoter from which it is transcribed. In the E. coli gal operon, expression of galE (epimerase) does not occur when the cistron is transcribed from the prophage lambda p_L promoter located upstream to the gal promoter. Expression of the promoter distal cistrons, galT (transferase) and galK (kinase) can be directed efficiently from λp_L. After surveying the proteins synthesized after lambda induction by two-dimensional gel electrophoresis, we have come to the conclusion that the absence of epimerase activity results from failure to translate the galE sequences in the p_L-gal transcript.

The identification of kinase and transferase was aided immeasurably by the analysis of a strain carrying a high copy number plasmid into which the gal operon had been cloned. The three products of the gal operon were readily apparent in two-dimensional gels of labelled extracts made from this strain, without D-galactose induction or prior purification. This approach, using cloned genomic fragments, should prove generally useful in identifying gene products.

The gal promoter region contains an extensive dyad symmetry which flanks the normal transcription start site. A transcript originating at p_L would include this symmetry. An RNA stem-loop structure with considerable stability (-20.7 kcal/mol) could form within the p_L-gal transcript, blocking the attachment of ribosomes to the translation initiation region galE. To assess the contribution of this symmetry to the failure of galE expression, we constructed a variety of p_L-gal fusion strains in which the formation of the stem-loop structure should be impaired or prevented. These strains include those bearing IS1 or IS2 insertions, deletions, or new promoters within the symmetrical region. In each of these cases, some expression of galE was observed after prophage induction, although in no case was the level of epimerase as high as when galE is expressed from its cognate promoter.

The data suggests that a stem-loop structure does play a role in the discoordinate expression of gal from p_L, but that other components are also involved, perhaps other symmetries between galE and the p_L-transcript. In the case of lambda cro-lac fusions, the efficiency of lac translation is influenced, in an apparently unpredictable way, by the sequences upstream of the lacZ translation initiation region. We cannot exclude alternative possibilities: 1. That ribosomes attached to sequences preceding galE might occlude the translation initiation region of galE. 2. The galE sequence of the p_L-transcript might be uniquely unstable.

Bacterial Genes in Mammalian Cells - Little is known about the mechanisms that enhance the integration of foreign genes into high molecular weight (hmw) DNA of eukaryotic cells and the expression there. Since evidence for integration as well as expression of E. coli derived genes in mammalian cells was provided by us earlier, we now tested for a possible enhancement of foreign gene expression in mouse cells after linkage of defined prokaryotic DNA to specific eukaryotic DNA sequences. Thus, an endonuclease restriction fragment from SV40 DNA, bearing the origin of replication of the viral DNA was linked to λdvgal 88 plasmid DNA, which carries part of the E. coli gal operon genes. This chimeric DNA was transferred together with herpes simplex virus type I thymidine kinase (TK) gene to

mouse TK^- cells. Analysis of the hmw DNA of TK^+ clones by DNA blotting and hybridization experiments provided evidence for the presence of prokaryotic as well as for SV40 DNA derived sequences. In addition, our results revealed enhanced integration of prokaryotic DNA sequences into cellular hmw DNA upon linkage to the SV40 signal. Two-dimensional electrophoresis indicated the expression of E. coli galactokinase in some of the transformants.

Animal Models - In a search for a possible host which can be used to study the E. coli galactose genes in vivo galactose metabolism was studied in D. melanogasters. Although Leloir enzymes could not be detected in D. melanogaster, galactose metabolism did occur. The Leloir enzymes may not be present. Thus, galactose metabolism may be due to an alternate pathway. If the Leloir enzymes are present in D. melanogaster, three different hypotheses may explain the findings. The Leloir enzymes in the fly extracts may have requirements which are not compatible with the enzyme assays which were employed. These assays have been used in systems ranging from bacteria to mammals, thus it is unlikely the enzyme assays are inadequate to detect the Leloir enzymes in D. melanogaster. Endogenous substances may be inhibiting the Leloir enzymes in the fly extracts. Since no Leloir enzymes were found in the fly extracts except for a trace amount of epimerase activity in the eggs, this possibility could not be tested. As an alternative, E. coli Leloir enzymes were used to test for inhibitors in the fly extracts. No inhibition of E. coli galactokinase or epimerase was found and there was only partial inhibition of E. coli transferase by adult, pupal and larval extracts. None of the E. coli Leloir enzymes were inhibited by the egg extract. This stressed assay for inhibition does not rule out the possibility of endogenous inhibitors of the Leloir enzymes in D. melanogaster. Finally, the fruit flies may maintain low levels of the Leloir enzymes which could not be detected. The enzyme assays would have to be 10 times as sensitive to rule out the possibility that Leloir enzymes may be converting galactose to CO_2. The assays employed for the Leloir enzymes are among the most sensitive assays in the literature.

Failure to detect the Leloir enzymes under one set of assay conditions cannot be taken as proof that the structural genes are absent in D. melanogaster. The enzyme hypoxanthine-guaninephosphoribosyl transferase (HGPRT) is not detectable in extracts of D. melanogaster larvae or adults.

Leloir pathway enzymes were also assayed in flies grown non-axenically. The data shows that even when flies were grown in the presence of bacteria and yeast only very low levels of kinase and epimerase activity appeared. High levels of Leloir pathway enzymes might have been expected due to the contaminating organisms. However, since the Leloir pathway enzymes are usually regulated in bacteria, such as E. coli, the contaminating organisms may not have been induced. However, the use of axenic cultures for studies of intermediate metabolism in D. melanogaster is strongly urged, as the presence of even trace amounts of enzymes from contaminating organisms can seriously confuse attempts to ascertain metabolic pathways.

The absence of galactokinase and galactose-1-phosphate uridyl transferase in D. melanogaster does not set a precedent in eukaryotes. Stephens (1974) found grey kangaroos which were missing both of these enzymes.

Publications:

Kamerow, H.N., Merril, C.R., and Nash, W.G.: Absence of galactose enzyme activities in axenic Drosophila melanogaster. Insect. Biochem. 11: 221-224, 1981.

Horst, J., Stanbro, H., and Merril, C.R.: On procaryotic gene expression in eukaryotic systems. Humangenetik 54: 289-302, 1980.

Merril, C.R., Gottesman, M.E., and Adhya, S.L.: E. coli gal operon proteins made after prophage lambda induction. J. Bact., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00936-18 LGCB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Homocystinuria: Methionine Metabolism in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB	NIMH
Other:	R. Havlik	Medical Officer, Epidemiology Branch	HV	NHLBI
	M. Feinleib	Chief, Epidemiology Branch	HV	NHLBI

COOPERATING UNITS (if any)

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LAB/BRANCH
Laboratory of General and Comparative Biochemistry

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Section on Alkaloid Biosynthesis

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3/12	3/12	0

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A survey of the health status of parents and grandparents of patients with homocystinuria due to cystathionine synthase deficiency has been completed. The results obtained indicate that mild homocyst(e)inemia is not an important contributory factor to atherosclerotic disease in the general population and provide no support for current hypothesis which postulate such an association.

Preliminary work has been initiated upon a questionnaire survey aimed at obtaining standardized information sufficient to provide insight into many presently unanswered questions as to clinical features of homocystinuria due to cystathionine β-synthase deficiency and the effects of various therapies now in use for this condition.

Project Description:

Since the last annual report, very limited time has been available to devote to this project. This reflects the gradual deemphasis on work in human genetic disease necessitated by the long-term attrition in manpower and facilities for this area of research, as mentioned in several previous annual reports. Accomplishments have been as follows:

(a) Completion of the report, described in detail last year, upon the cardiovascular risk in obligate heterozygotes for homocystinuria due to cystathionine β-synthase deficiency.

(b) Preparation of a revised chapter upon "Disorders of Transsulfuration" for a new edition of the book edited by J.B. Stanbury, J.B. Wyngaarden, and D.S. Fredrickson, The Metabolic Basis of Inherited Disease.

(c) Initiation of work on a questionnaire survey to obtain information upon patients with homocystinuria due to cystathionine β-synthase deficiency. The aim is to obtain sufficient information to gain insight into a variety of presently unanswered questions. For example: Are there clinical differences between pyridoxine responsive and non-responsive patients? If so, what are they? What is the rate of occurrence of thromboembolic episodes in each subgroup? What is the frequency of dislocated lenses as a function of patient age? How many of these patients are mentally retarded? Are there individuals with this enzyme deficiency who are clinically normal? Above all, what therapies are now being used, and what is their real impact?

We will try to obtain information from appropriate physicians about each of the 350-500 homocystinuric patients who have been identified worldwide. The cooperation and ideas of a number of key physicians has already been secured for this project. Work is presently at an early stage and we are awaiting OMB clearance of the questionnaire form.

Publications:

Mudd, S.H., Ebert, M.H., and Scriver, C.R.: Labile methyl group balances in the human: the role of sarcosine. Metabolism 29: 707-720, 1980.

Schulman, J.D., Mudd, S.H., Schnider, J.A., Sheetz, M., Spielberg, S.P., Boxer, L.A., Oliver, J., and Corash, L.M.: Inborn errors of glutathionine and sulfur amino acid metabolism. Ann. Intern. Med. 93: 330-346, 1980.

Mudd, S.H., Havlik, R., Levy, H.L. McKusick, V.A., and Feinleib, M.: Lack of excess heart attack or stroke risk in heterozygotes for homocystinuria due to cystathionine synthase deficiency. Am. J. Human Genet., in press.

Mudd, S.H., and Levy, H.L.: Disorders of Transsulfuration. In Stanbury, J.B., Wyngaarden, J.B., and Fredrickson, D.S. (Eds.): The Metabolic Basis of Inherited Disease, 5th Ed. New York, McGraw-Hill, in press.

Schulman, J.D., Mudd, S.H., Shulman, N.R., and Landvater, L: Pregnancy and thrombophlebitis in pyridoxine nonresponsive homocystinuria associated with cystathionine synthase deificiency. Blood 56: 326, 1980.

Cogan, D.G., Schulman, J.D., Porter, R.J., and Mudd, S.H.: Epileptitorm eye movements with methylmalonic aciduria and homocystinuria. Case report. Am. J. Ophthalmol. 90: 251-253, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00937-16 LGCB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Transsulfuration in Higher Plants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Giovanelli	Research Chemist	LGCB NIMH
Other:	S. H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB NIMH
	A. H. Datko	Botanist	LGCB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Alkaloid Biosynthesis

INSTITUTE AND LOCATION

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3	1	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In previous estimations of the relative syntheses of the sulfur and 4-carbon moieties of methionine (made with separate cultures of Lemna), effects of biological variation could not be totally excluded. This problem has now been overcome by determination of these syntheses in the same culture of Lemna. It was confirmed that methionine inhibits de novo synthesis of its sulfur moiety more than its 4-carbon moiety, thereby showing that methionine is synthesized by a pathway (termed "preferential methionine sulfur recycling") in addition to the established one through transsulfuration. The spermidine content of Lemna is about 1/2 of the amount of methionine metabolized through preferential methionine sulfur recycling, and it is suggested that a physiological role for this cycle in plants is to conserve methionine sulfur and prevent the accumulation of methylthioadenosine formed during polyamine synthesis. It was further shown that transmethylation is the dominant pathway for methionine metabolism, accounting for 4 to 5 times the amount of methionine accumulating in protein. These are the first quantitative estimates in a higher plant of the relative extent of methionine metabolism through major pathways.

Project Description:

The primary goal of this project is to elucidate the patterns of control of methionine biosynthesis and metabolism in Lemna. Previous experiments clearly established that exogenous methionine inhibits incorporation of sulfate sulfur into cystathionine and its products without affecting that into cysteine, suggesting that methionine inhibits its own synthesis at the cystathionine synthase step. Based on our understanding at the time that de novo synthesis (from sucrose) of the 4-carbon moiety of methionine proceeds exclusively via transsulfuration (i.e., cystathionine synthesis), it was argued that exogenous methionine should inhibit incorporation of the 4-carbon moiety to the same extent. Experiments to test this prediction showed surprisingly that incorporation of the 4-carbon moiety was reduced to only a small extent by exogenous methionine. However, these experiments were not considered definitive, since different Lemna cultures were used in determining incorporation of the sulfur and 4-carbon moieties of methionine, and the possibility that the results could be explained by biological variation could not be totally excluded.

We have now determined the relative de novo synthesis of the sulfur (from sulfate), 4-carbon (from sucrose) and methyl (from sucrose) moieties of methionine in the same culture of Lemna. This determination was made by following the changes in the relative amounts of radioactivity in these three moieties when exogenous [^{35}S,U-^{14}C]-methionine at concentrations of 0.67 μM and 2 μM was incorporated into protein methionine. Special methods were developed to assay radioactivity in the three moieties. The results of these experiments confirm our previous findings that methionine inhibits de novo synthesis of its sulfur moiety more than that of its 4-carbon moiety, and have further provided, for the first time in higher plants, quantitative estimates of major pathways for methionine metabolism. Demonstration of unequal inhibition of the de novo synthesis of sulfur and 4-carbon moieties of methionine shows that methionine must be synthesized by a second pathway in addition to the established one through transsulfuration. The amount of methionine metabolized through this pathway (termed "preferential methionine sulfur recycling") amounts to about 1/3 of that accumulating in protein. By analogy with studies in other laboratories with animals, yeast and ripening apples, it is proposed that the cycle proceeds as follows: methionine → S-adenosylmethionine → methylthioadenosine → methionine. Preferential methionine sulfur recycling has been previously reported in plants only in ripening apples which are unusual in metabolizing methionine predominantly to ethylene (and stoichiometric amounts of methylthioadenosine). Operation of preferential methionine recycling in Lemna, which was shown to evolve little or no ethylene, suggests that this pathway may be of wide distribution and play a fundamental role in plants. The spermidine content of Lemna was found to be approximately 40-50% of the amount of methionine metabolized through preferential methionine sulfur recycling, and it is tentatively suggested that a physiological role for this cycle in plants is to conserve methionine sulfur and prevent the accumulation of methylthioadenosine formed during polyamine synthesis. The discovery of this cycle in Lemna is an important advance in our studies of control of methionine biosynthesis. It is now clear that a pathway for methionine biosynthesis in addition to the established route via transmethylation is operating in Lemna, and that an understanding of the reactions of this pathway and their control will be required to provide a comprehensive picture of the patterns of regulation of methionine biosynthesis.

These experiments also showed that transmethylation is the dominant pathway of methionine metabolism, accounting for 4 to 5 times the amount of methionine accumulating in protein. The methyl groups of phosphatidylcholine account for at least 40% of the methionine metabolized through transmethylation.

Future work will be continued along the following lines:

(1) The results described for metabolism of the sulfur, 4-carbon and methyl moieties were determined with Lemna growing in concentrations of methionine that increase the pool sizes of S-adenosylmethionine and soluble methionine by one and two orders of magnitude, respectively. These experiments will be extended to plants growing in the presence of a tracer concentration of methionine that does not significantly increase the pool sizes of these amino acids. Comparison of results from these two sets of experiments should answer two important questions. First, the extent to which methionine (or a derivative such as S-adenosylmethionine) controls preferential methionine sulfur recycling. Second, whether methylneogenesis in plants is regulated in vivo by methionine (or a derivative).

(2) The pathway of preferential methionine sulfur recycling will be elucidated. Since methylthioadenosine and methylthioribose are suspected intermediates, we will first determine by nutritional and isotope experiments whether these compounds are converted to methionine.

(3) The experiments described above have provided the first quantitative estimates in plants of the relative amounts of methionine metabolized in vivo through the major pathways such as incorporation into protein, polyamine synthesis, and transmethylation. The various pathways through which methionine is metabolized will be defined in more detail by use of methionine labeled specifically in the appropriate moiety, and the quantitative fluxes through these pathways determined.

(4) The findings in the accompanying report by Thompson indicate that regulation of cystathionine synthase by itself is not sufficient to control the flux through the cystathionine synthase step, and that an additional regulatory element, such as the allosteric stimulation by S-adenosylmethionine of threonine synthase, is required. The regulatory properties of threonine synthase from Lemna will be determined, and their possible relationship to the control of methionine biosynthesis investigated.

Publications:

Giovanelli, J., Mudd, S.H., and Datko, A.H.: Sulfur amino acids in plants. In Stumpf, P.K., and Conn, E.E. (eds.): The Biochemistry of Plants: A Comprehensive Treatise, Chap. 12. New York, Academic Press, 1980, Vol. V, pp. 454-505.

Macnicol, P.K., Datko, A.H., Giovanelli, J., and Mudd, A.H.: Homocysteine biosynthesis in green plants: Physiological importance of the transsulfuration pathway in Lemna paucicostata. Plant Physiol., in press.

Giovanelli, J., Mudd, S.H., and Datko, A.H.: Recycling of methionine sulfur in a higher plant by two pathways characterized by either loss or retention of the 4-carbon moiety. Biochem. Biophys. Res. Commun. 100: 831-839, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 MH 00938-02 LGCB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Effect of B_{12} on Folate Uptake, Utilization and Interconversion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: B. W. Uhlendorf Research Microbiologist LGCB NIMH

COOPERATING UNITS (if any)

None

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Section on Proteins

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5/12	5/12	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project discontinued.

Project Description:

Project discontinued due to resignation.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00939-01 LGCB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Enzymes of Methionine Biosynthesis in Higher Plants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. A. Thompson	Senior Staff Fellow	LGCB NIMH
Other:	S. H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB NIMH
	A. H. Datko	Botanist	LGCB NIMH

COOPERATING UNITS (if any)

None

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Laboratory of General and Comparative Biochemistry

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1-6/12	1-5/12	1/12

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of the enzymes of methionine biosynthesis in Lemna revealed the following: (a) O-Acetylserine sulfhydrylase has a 1000-fold higher V_{max} and a 70-fold lower K_m for sulfide than O-phosphohomoserine sulfhydrylase, providing a kinetic basis for the dominance of transsulfuration in homocysteine synthesis. (b) Feeding propargylglycine (PAG), a suicide inhibitor of cystathionine γ-synthase to Lemna showed that only 16% of control synthesis activity is required to maintain normal methionine synthesis. Therefore, the 85% reduction in synthesis activity observed in the presence of methionine is not sufficient, by itself, to regulate the pathway. (c) Further studies with PAG showed that plants with less than 10% of control cystathionine γ-synthase activity were not viable in the absence of other factors. Addition of 31 μM L-cystine permitted growth with 9% normal activity probably because internal concentrations of L-cysteine, a cosubstrate for the synthesis, were elevated. (d) L-Cysteine is an irreversible inhibitor of cystathionine γ-synthase. Whether the inhibition is important in vivo requires further study; plants grown in 31 μM L-cystine have only 60% of the control enzyme activity.

Project Description:

A major objective of the research in this laboratory is to evaluate the mechanism of control of methionine biosynthesis in higher plants. As one approach, specific inhibitors of various steps in the pathway have been developed which impair methionine biosynthesis in our model plant, Lemna paucicostata. Such inhibitors are especially valuable because they can be used as tools to study whether or how plants adapt to conditions of limiting methionine.

One method of evaluating any such adaptation is to measure change in the activity of key enzymes in the methionine pathway. In accord with this approach, I have been studying the effect of methionine excess and limitation on the activities of cystathionine γ-synthase and O-phosphohomoserine sulfhydrylase. Cystathionine γ-synthase catalyzes reaction 1, the committing step in methionine biosynthesis.

$$\text{O-phosphohomoserine} + \text{cysteine} \rightarrow \text{cystathionine} + P_i \qquad (1)$$

O-Phosphohomoserine sulfhydrylase catalyzes reaction 2, a potential alternative pathway for homocysteine biosynthesis.

$$\text{O-phosphohomoserine} + \text{sulfide} \rightarrow \text{homocysteine} + P_i \qquad (2)$$

The activity of both enzymes increase two- to three-fold under conditions of methionine limitation and decrease five- to six-fold when external methionine is supplied. Thus, both activities appear to be regulated by methionine in such a way as to suggest that they are properties of one enzyme. Pursuant to these findings, the following studies were undertaken.

(1) Previous studies have shown that at least 90 to 95% of homocysteine formed in vivo comes from cystathionine (transsulfuration) and have failed to detect any homocysteine formation via the O-phosphohomoserine sulfhydrylase reaction (direct sulfhydration). Considering that each of the necessary enzyme activities and the corresponding substrates for both pathways are present, the failure to detect homocysteine synthesis by direct sulfhydration has been puzzling. By measuring the kinetic constants for each of these reactions and also for reaction 3, catalyzed by O-acetylserine sulfhydrylase,

$$\text{O-acetylserine} + \text{sulfide} \rightarrow \text{cysteine} + \text{acetate} \qquad (3)$$

it was determined that (a) whereas the V_{max} and K_m for O-phosphohomoserine in the cystathionine γ-synthase and O-phosphohomoserine sulfhydrylase reactions were similar, the K_m for cysteine in the synthase reaction was 7-fold lower than the K_m for sulfide in the O-phosphohomoserine sulfhydrylase reaction, (b) the K_m for sulfide in the O-acetylserine sulfhydrylase reaction was 70 times lower than the corresponding K_m in the O-phosphohomoserine reaction, and (c) the V_{max} for the O-acetylserine sulfhydrylase reaction was 1000 times greater than that for the O-phosphohomoserine sulfhydrylase reaction. Taken together with available information on the amounts of O-phosphohomoserine and O-acetylserine present, these data suggest that virtually all of the available sulfide will go through reaction 3 to cysteine rather than directly to homocysteine (reaction 2) and that homocysteine formation by transsulfuration proceeds at greater than 800 times the rate by the alternative direct sulfhydration pathway.

(2) One of the inhibitors of methionine biosynthesis that is being investigated in our laboratory, propargylglycine, is a potent suicide inhibitor of cystathionine γ-synthase. Preincubation of an extract with 5 μM DL-propargylglycine destroyed 50% of the enzyme activity within 10 min. The inactivation was completely prevented by the presence of either 20 μM O-phosphohomoserine or O-succinylhomoserine, but not by cysteine. Furthermore, activity was not restored by either gel filtration or by addition of pyridoxal phosphate.

Two particularly useful aspects of a suicide inhibitor, in vivo, are that (a) it is very specific in its action since the inhibitor must not only bind to the target enzyme but also must be acted upon by the enzyme as if it were a substrate and (b) the inhibition is irreversible so that the amount of activity measured in an extract of the tissue to which the inhibitor has been administered is an accurate reflection of the amount of activity that was present in vivo. By giving propargylglycine to Lemna, it was found that its effect on enzyme activity was rapid (88% inhibition within 2 h at 40 μM DL-propargylglycine) and potent (0.5 μM DL-propargylglycine for 18 h inhibits 70% of the activity). By measuring the amount of cystathionine γ-synthase activity at the highest concentration of propargylglycine that would permit normal growth, it was discovered that in Lemna normal growth and methionine biosynthesis were maintained when only 16% of the normal enzyme activity was present. By increasing the dose of propargylglycine, it was found that at least 10% of the normal amount of activity was required for survival, even at diminished growth rates. Considering that 15 to 20% of the normal amount of cystathionine γ-synthase activity still remains when external methionine is present, the results with propargylglycine suggest that other factors in addition to the repression of cystathionine γ-synthase are involved in regulating this pathway.

(3) In performing the in vivo propargylglycine experiments, it was found that the presence of 31 μM L-cystine in the medium could rescue plants from a normally lethal dose (150 μM) of DL-propargylglycine. An investigation of the amount of enzyme in plants grown at sublethal (10 and 40 μM) DL-propargylglycine, with and without 31 μM L-cystine showed that the presence of cystine in the growth medium had no appreciable effect on enzyme activity. At 150 μM DL-propargylglycine, and 31 μM L-cystine, the plants had only 9% of the normal enzyme activity even though they grew as if they had almost 12% when no cystine was present. In the absence of cystine, plants with only 9% of normal activity are not viable. Thus, the data suggest that the cystine rescue is the result of an estimated 30% increase in internal cysteine concentration which, as a cosubstrate for cystathionine γ-synthase, is sufficient to boost methionine synthesis to a level which permits growth.

(4) Cystathionine γ-synthase activity in Lemna grown in 31 μM L-cystine (and no propargylglycine) was only 60% of that in control plants. It is possible that an increase in internal cysteine leads to elevated methionine synthesis which in turn partially represses synthase activity. However, in vitro experiments in which extracts were pre-incubated with cysteine before being assayed also showed diminished activity when compared with samples of the same extract which were pre-incubated without cysteine. Pre-incubation with 1 mM L-cysteine caused a 50% inactivation of the enzyme within 10 min at 30°. The inactivation could not be reversed by gel filtration or by addition of DTT although the presence of 32 mM DTT does afford some protection ($t_{1/2}$ = 25 min). O-Succinylhomoserine (40 mM)

also protects to some extent ($t_{1/2}$ = 17 min with 10 mM L-cysteine compared to $t_{1/2}$ = 5 min in 10 mM L-cysteine without O-succinylhomoserine). The exact mechanism for the cysteine inactivation is not understood. Whether this phenomenon is related to the decreased activity detected when cystine is administered in vivo remains to be clarified.

Future work will be continued along the following lines:

(1) Propargylglycine, by inhibiting cystathionine γ-synthase, brings about a state of methionine deprivation in Lemna. As such, this should lead to a derepression of the synthase. Growth experiments show that plants exposed to 40 nM DL-propargylglycine undergo a transient period between 24 and 48 hours after administration of the inhibitor during which no new fronds appear and after which growth resumes at a slower than normal rate. Attempts to correlate the recovery with derepression of cystathionine γ-synthase are presently underway.

(2) The molecular mechanism for control of cystathionine γ-synthase activity will be studied. Questions that will be examined include: (a) Does the enzyme derepress if conversion of methionine to S-adenosylmethionine is blocked by specific inhibitors of methionine adenosyltransferase? (b) Does the enzyme derepress under SO_4^{2-} starvation (or in the presence of MoO_4^{2-}) when plants are limited for both cysteine and methionine? If not, is cysteine required as a positive regulator? (c) Do inhibitors of protein synthesis, e.g., cycloheximide, etc., prevent synthesis of new enzyme and thus block the observed derepression phenomenon?

(3) In studying the mechanism of regulation of cystathionine γ-synthase, it would be valuable to know if the amount (as opposed to activity) of the enzyme increases. It would therefore be useful to purify cystathionine γ-synthase to use as a marker on polyaceylamide gels or to make specific antibodies. As a side benefit of having purified enzyme, it would be possible to characterize the physico-chemical properties of this enzyme.

(4) It would be valuable to know if other enzymes in the pathway are affected by methionine. Thus, similar experiments to those described in this and in last year's report are planned for β-cystathionase. The availability of aminoethoxyvinylglycine, a potential suicide inhibitor of this enzyme, may provide a tool which, by analogy with propargylglycine, can be used to determine the relationship between enzyme activity and flux through this step of the pathway.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00940-01 LGCB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Methionine Biosynthesis in Higher Plants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To study the mechanisms which regulate methionine biosynthesis, a search was made for inhibitors of specific steps in the pathway using Lemna as the experimental model. Nutritional uptake and labeling experiments were used to determine the site, extent and specificity of the metabolic block brought about by each inhibitor. A non-steady-state uptake experiment showed that molybdate interfered with the uptake of sulfate but provided no evidence of further impairment in organification of sulfate. Prevention of molybdate growth inhibition by cystine but not cystathionine or methionine provided support for our previous conclusion that transfer of sulfur from homocysteine to cysteine does not occur in plants. The metabolic block effected by lysine plus threonine was localized to the conversion of aspartate to homoserine. Propargylglycine produced growth inhibition due to inhibition of cystathionine γ-synthase activity. Growth in aminoethoxyvinylglycine caused impaired cleavage of cystathionine to homocysteine. These results establish that each of these inhibitors brings about a methionine limitation, and thus validates their use in the study of regulation of the methionine pathway.

Project Description:

The mechanisms which regulate methionine biosynthesis in plants have not been elucidated. To study this problem we have searched for inhibitors of specific steps in the pathway, using Lemna (duckweed) as the experimental material. It was hoped that each inhibitor would bring about methionine limitation thereby providing an experimental tool for study of regulation of the pathway.

Our earlier nutritional experiments with the inhibitors chosen for this study (see previous annual report, Z01 MH 00937-15, 1980) indicated that each caused growth inhibition of Lemna and that the inhibition could be prevented in each instance by addition of intermediates after the putative metabolic block. In several instances compounds preceding the block also prevented growth inhibition. To verify the nutritional results concerning the site affected and to measure the extent of inhibition caused by each inhibitor, uptake and labeling experiments were performed.

As concerns inhibition by molybdate, this anion was shown to interfere with uptake of sulfate in a non-steady-state labeling experiment using $^{35}SO_4^{2-}$. When molybdate was present in the medium at a molybdate to sulfate ratio of 20:1, molybdate blocked sulfate uptake by 90% within an hour. The portion of radioactivity taken up which became organic was the same in the presence or absence of molybdate. This result suggests that molybdate did not affect organification of sulfate, but because the inorganic sulfate pool was probably falling rapidly, unequivocal interpretation of this observation is not possible. A labeling experiment in which Lemna was grown to isotopic steady state in the presence of $^{35}SO_4^{2-}$, with molybdate present at a molybdate to sulfate ratio of 2.6:1, showed that such plants indeed had a lowered sulfate pool. Unexpectedly, however, their soluble sulfur amino acids were increased over that found in control (molybdate to sulfate 0.3:1) plants.

As concerns prevention of molybdate growth inhibition by cystine but not by methionine or cystathionine, molybdate (in a 20:1 ratio with sulfate) was shown not to interfere with the uptake of methionine. The failure of methionine to sustain growth indicates that transfer of sulfur from methionine to cysteine does not occur in Lemna at a rate sufficiently high to meet the plants' requirement for growth. The failure of cystathionine to sustain growth strongly suggests that Lemna lacks γ-cystathionase activity, in agreement with our previous failure to demonstrate activity of this enzyme in extracts of other plants. Thus, transsulfuration in the direction of homocysteine to cysteine is not likely to be an important metabolic pathway in plants.

The site of the metabolic block brought about by the combination of lysine plus threonine was localized to the conversion of aspartate to homoserine, the immediate precursor of phosphohomoserine (the source of the 4-carbon moiety of methionine). In a labeling experiment in which Lemna was grown to isotopic steady state in the presence of $^{35}SO_4^{2-}$, lysine, threonine and methionine, the accumulation of $^{35}SO_4^{2-}$ into cystathionine and its products was decreased when compared to plants grown in $^{35}SO_4^{2-}$ only or in $^{35}SO_4^{2-}$ and methionine. Cystathionine itself became virtually undetectable. Uptake experiments using labeled amino acids showed that whereas at lower concentrations of lysine plus threonine, methionine may have been preventing growth inhibition by preventing uptake of

lysine or threonine or both, at higher lysine plus threonine concentrations, methionine and cystathionine were acting as metabolite replacements when tested in nutritional experiments.

The reduced sulfur and aspartate pathways converge with the formation of cystathionine in a reaction catalyzed by cystathionine γ-synthase. Propargylglycine-induced growth inhibiton of Lemna was due to inhibition of this enzyme activity. Conversion of $^{35}SO_4^{2-}$ to cystathionine and its products was virtually undetected in plants grown in $^{35}SO_4^{2-}$, propargylglycine and methionine. Uptake studies showed that prevention of the inhibition by methionine, cystathionine, and cystine was not due to interference with propargylglycine uptake, whereas prevention by aspartate may well have been. Prevention by cystine, which preceeds the metabolic block, is understandable if medium cystine increased tissue cysteine. Even a small portion of uninhibited γ-synthase activity could then promote sufficient flux through this step to permit growth.

Cystathionine is cleaved to homocysteine (the immediate precursor of methionine) by β-cystathionase. Aminoethoxyvinylglycine (AVG) produced growth inhibition of Lemna. The metabolic site affected was β-cystathionase. A labeling experiment showed that plants grown in $^{35}SO_4^{2-}$, AVG and methionine accumulated 35-fold more cystathionine than did control plants. Uptake experiments showed that all amino acids tested interfered somewhat with AVG uptake. Since a very steep concentration dependence was found for AVG growth inhibition, it was not possible to establish if prevention of growth inhibiton was due to uptake interference, metabolite replacement, or both.

Future work with these inhibitors will concern the following:

(1) There is reason to believe, both on enzyme-mechanistic grounds and from the nutritional results, that propargylglycine and AVG may be affecting sites other than those mentioned above. AVG is known to also inhibit ACC synthase, the enzyme which converts S-adenosylmethionine to 1-aminocyclopropane-1-carboxylic acid, the immediate precursor of the hormone ethylene. Experiments are planned to examine if this, or other pathways are inhibited by this compound.

(2) It has been found that Lemna can adapt to growth in the presence of sub-lethal concentrations of at least some of the inhibitors described above. (a) At intermediate molybdate to sulfate ratios (i.e., 2.6:1), Lemna plants shown the morphological characteristics typical of sulfur limitation and exhibit a growth rate slower than that of control plants. Both of these characteristics can be maintained for many days in subculture. The labeling experiment described above showed that these plants had a lowered inorganic sulfate pool. Surprisingly, however, accumulation of soluble sulfur amino acids was increased. This phenomenon will be examined more closely. (b) Lemna can be grown for extended periods in low concentrations of propargylglycine, and of lysine plus threonine. In each case, the plants show a virtual cessation of frond emergence which can be seen within a few hours of exposure to the inhibitor. Some 24 hours later frond emergence is resumed, but at a rate somewhat slower than that shown by control plants. Upon subculture in the same medium no further transient is observed and the new slower growth rate is maintained. Upon subculture of adapted plants to medium with a higher inhibitor concentration, again a transient is observed. "Naive" plants under these latter conditions would be killed. Both

of these inhibitors will be studied in hopes of defining the metabolic basis of adaptation.

(3) A search will be made for inhibitors which interfere with the conversion of methionine to S-adenosylmethionine. The latter compound has been proposed to be a major effector in the control of methionine biosynthesis in plants. Several compounds known to inhibit S-adenosylmethionine synthesis in other tissues will be examined for their effects on Lemna growth. Appropriate uptake and labeling experiments will be carried out in conjunction with these nutritional experiments.

Publications:

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Macnicol, P.K., Datko, A.H., Giovanelli, J., and Mudd, A.H.: Homocysteine biosynthesis in green plants: Physiological importance of the transsulfuration pathway in Lemna paucicostata. Plant Physiol., in press.

Giovanelli, J., Mudd, S.H., and Datko, A.H.: Recycling of methionine sulfur in a higher plant by two pathways characterized by either loss or retention of the 4-carbon moiety. Biochem. Biophys. Res. Commun. 100: 831-839, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00941-01 LGCB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biochemical Genetics and Metabolic Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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3	1	2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Recently developed sensitive silver stains allow detection of trace proteins. Computerized microdensitometry has added a third dimension, quantitation to the two-dimensional electrophoretograms. These techniques were used to examine the Lesch-Nyhan syndrome. Four hundred lymphocyte proteins were analyzed on each electrophoretogram. Quantitative differences were found in the lymphocyte autoradiograms. Eleven which were significantly different at the $2P < .01$ level. This study indicates that characteristic secondary changes in proteins may be observed, permitting the establishment of a catalogue of protein changes in metabolic diseases.

This technology has also been used to study proteins affected by chromosome 21, the chromosome associated with Down's syndrome. A quantitative correlation has been found in gel analysis between the protein superoxide dismulate, coded for by chromosome 21, and the number of chromosome copies per cell. Location of the galactose operon gene products from E. coli in 2-D electrophoretograms are allowing us to monitor human cells containing E. coli genes in various vectors.

Disorders of the Central Nervous System - Human behavioral disorders are particularly difficult to investigate at the molecular level. There are no trait specific molecular markers for diseases of the central nervous system such as schizophrenia, Huntington's disease, Alzheimer's disease and manic-depressive illness. The Lesch-Nyhan syndrome is one of the few neuropsychiatric diseases for which molecular details are available (1). For this reason it was chosen as a model system to find markers and uncover pathophysiologic protein changes with quantitative two-dimensional electrophoretic techniques.

Proteins from patients with Lesch-Nyhan syndrome and control individuals were studied using enzymatic, immunologic and two-dimensional electrophoretic techniques. Four hundred proteins were analyzed on each two-dimensional electrophoretogram for positional or quantitative variation. In autoradiograms of phytohemaglutinnin stimulated, ^{14}C-labelled lymphocytes, there were eleven quantitative differences that were significant at the $2P < 0.01$ level. A significant quantitative difference was also found in an analysis of silver stained gels of unstimulated lymphocytes. Immunologic and enzymatic studies of hypoxanthine-guanine phosphoribosyltransferase, the enzyme which is defective in the Lesch-Nyhan disease, established that while antigen capable of cross-reacting with antibody made to normal HPRT was absent, there was still a nearly normal amount of HPRT protein present in two-dimensional gels and trace amounts of HPRT activity in erythrocyte lysates of the three patients studied.

Currently the technology of two-dimensional electrophoresis can detect only a small fraction of the total cellular proteins and it is not always possible to electrophoretically demonstrate an alteration in a defective protein. However, specific secondary changes in other polypeptides might be observed. The establishment of a catalogue of protein changes in metabolic diseases would serve as an aid in diagnosis and in understanding the associated pathophysiology.

A larger clinical study of protein markers in Huntington's disease is nearing completion. Lymphocytes, erythrocytes, plasma and CSF obtained from 30 individuals studied clinically on our ward have been obtained, electrophoresed and the patterns computerized. This group includes 13 patients with Huntington's disease, two individuals at risk, neurological controls with Shy-Drager's disease, Alzheimer's disease and neurofibromatosis and elderly controls. Protein variations in Huntington's disease are being catalogued against the background range of normal variations in protein charge, molecular weight and density.

More than 300 protein species were detected in 60 µl of human CSF. A 'map' of the normal human pattern was established, with 26 proteins identified and 10 clusters of differences between plasma and CSF protein patterns indicated. In primates, large qualitative differences were found between ventricular, cisternal and lumbar CSF proteins. This work was facilitated by continued development of the silver stain methods.

A preliminary study of two-dimensional electrophoretic protein patterns from patients with Alzheimer's disease yielded complex patterns which were beyond our ability to analyze at that time. In an effort to narrow the analysis, it was recognized that patients with an extra copy of chromosome 21 developed Down's syndrome and if they live long enough Alzheimer's disease. For these reasons, a search was begun for proteins whose expression is affected by chromo-

some 21. The protein spot corresponding to SOD_S, which is coded for by chromosome 21, was located by co-electrophoresis of purified enzyme with cellular proteins. The density of the SOD_S was measured in fibroblast protein extracts from cells which are trisomic, disomic, and monosomic for chromosome 21. The densities on silver stained gels and autoradiograms, each prepared from samples extracted by different methods, were measured. Normalized density ratios closely approximated the previously reported enzyme activity ratios of 1.5:1.0:0.5 for trisomic, disomic, and monosomic cells.

The technique of double labelling was utilized to search for proteins coded for by human chromosome 21 or influenced by its presence in a human-mouse hybrid cell line. No clear examples other than SOD_S were found by double labelling in this study. Our inability to detect such proteins by double labelling could be due to insufficient production of human protein, production of human proteins which are not observed under the electrophoresis conditions employed in this study, or co-migration with mouse proteins. It has been estimated that 50% of human and mouse proteins co-migrate on two-dimensional gels. However, another double labelling study using a Chinese hamster-human cell hybrid line demonstrated proteins which were influenced by the presence of human chromosome 21. The Chinese hamster cell may be sufficiently different from the mouse cell to allow detection of differential protein expression with the double label technique.

Quantitative analysis of the proteins of human-mouse hybrid cells and mouse parental fibroblasts showed one protein spot to have twice the density in the human-mouse hybrid line. This protein could be a gene product coded for by chromosome 21, and be of such composition that it co-migrates with an identical protein in the mouse. Alternatively, this could be a mouse protein whose quantity is affected by the presence of human chromosome 21.

Two additional differences were found between the human-mouse hybrid cell and mouse cell proteins. Both are possible charge shifts. However, the second of these may be a more complex alteration since the silver stained protein is also more dense in human-mouse hybrid cells. The protein which has an apparent charge shift was observed only in silver stained gels. In this case, double labelling was not capable of illustrating an effect of the presence of human chromosome 21. In general, double labelling relies on qualitative differences, while methods employed in this study are capable of detecting quantitative differences as well and may detect human proteins which co-migrate with mouse proteins.

This study illustrates the need for multiple technologies to search for proteins whose expression is influenced by the presence of human chromosomes in somatic cell hybrids. Delineation of proteins affected by chromosome 21 may lead to a better understanding of the pathophysiology associated with Down's syndrome.

Development of Genetic Models for Effects of Alcohol - Mice selected for a differential sensitivity to the soporific effects of alcohol by Dr. Deitrich and coworkers (University of Colorado) show a 25-fold difference in sleep time. This is correlated with a region specific (cerebellar vermis vs. hippocampus) difference in the cellular sensitivity to ethanol, and this difference may be cell type specific. Since these two strains appear to be genetically fixed for their difference in alcohol sensitivity, it seems reasonable to look for differential gene expression in the cerebellar vermis that is correlated with sleep time. To

do this, we are using the technique of high resolution two-dimensional electrophoresis coupled with a sensitive silver stain to separate and specially identify most polypeptide components of the vermis. In addition, strain-specific differences in the quantity, isoelectric point, and molecular weight of these polypeptides are being catalogued with the aid of computer analysis.

Thus far, 1042 polypeptides from cerebellar vermis have been cataloged. Of these, 767 are in a region of the IEF dimension (ca. pH 4.5-7.5) that results in two-dimensional images of sufficient precision to analyze for qualitative differences. Seventeen stain specific differences have been identified that are invariant from mouse to mouse within a given strain. Of the eight differences between the short sleep and long sleep mice, only two still correlated with sleep time difference when images from C57BL/6J and DBA/2J mice were examined. There is a high molecular weight (>100,000) "family" with three isoelectric point species that shift in unison anodically in long sleep and DBA/2J and cathodically in short sleep and C57BL/6J. These represent the two differences that correlate with sleep time among all strains when assumptions of relatedness are not made.

Application to Cell Transformation - The modulation of gene expression accompanying neoplastic transformation has been assessed by computerized microdensitometry of autoradiographic patterns of ^{35}S-methionine labelled polypeptides separated by two-dimensional polyacrylamide gel electrophoresis. Nearly 1000 polypeptide species of parent diploid human fibroblasts (KD strain) and clonally-derived malignant fibroblasts (HUT-14 strain) were compared. HUT-14 fibroblasts express a mutation in one of the two functional β-actin genes and possess properties that distinguish them as neoplastic cells. Of the 700 more abundant polypeptides measured, 13 were lost and 14 were gained following this neoplastic transformation. It is estimated that 2%, or less, of the genes expressing abundant polypeptides were either activated or shut off, but at least 32% were modulated quantitatively as a consequence of this neoplastic transformation. Classes of "highly variable" and "marginally variable" polypeptides were assigned. Among the "highly variable" polypeptides, two related species barely detectable in KD parental cells were synthesized at a 25- to 31-fold higher rate in the transformed cells and the cell-associated and extracellular matrix forms of fibronectin were each diminished by more than 90%. Principles emerging from this study may form a basis for interpretation of the role of individual genes in the expression of neoplastic characteristics in HUT-14 cells.

Publications:

Goldman, D., Merril, C.R., and Ebert, M.H.: Two-dimensional gel electrophoresis of cerebrospinal fluid proteins. Clin. Chem. 26: 1317-1322, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00981-16 LNB

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Analysis of the Macromolecular Structure of Nerve Membrane During Excitation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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SUMMARY OF WORK (200 words or less - underline keywords)

The mechanism of nerve excitation was studied from a macromolecular viewpoint. During excitation of a crab nerve and a squid giant axon, the membrane surface was found to exhibit a small, rapid outwards movement. Simultaneously, the pressure detected with a detector pressing the membrane surface was shown to rise rapidly. These newly discovered phenomena are regarded as a sign of swelling of the axolemma-ectoplasm complex during the action potential. By using pyroelectric polymer material, polyvinylidene fluoride, faithful records of temperature changes associated with excitation of a crab nerve were obtained.

Publications:

Iwasa, K., and Tasaki, I.: Mechanical changes in squid giant axons associated with production of action potentials. Biochem. Biophys. Res. Commun. 95: 1328-1331, 1980.

Tasaki, I., Iwasa, K., and Gibbons, R.C.: Mechanical changes in crab nerve fibers during action potentials. Jpn. J. Physiol. 30: 897-905, 1980.

Tasaki, I.: Optical changes during electrogenesis. Adv. Physiol. Sci. 4: 163-170, 1980.

Tasaki, I., and Iwasa, K.: Swelling of nerve fibers during action potentials. Upsala J. Med. Sci. 85: 211-215, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00983-03 LNB

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biochemical Studies on the Mechanism of Nerve Excitation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Baumgold	Senior Staff Fellow	LNB NIMH
	P. Gallant	Staff Fellow	LNB NIMH
	I. Zimmerman	Biological Tech.	LNB NIMH

COOPERATING UNITS (if any)

Marine Biological Laboratory
Woods Hole, MA

LAB/BRANCH

Laboratory of Neurobiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Proteins involved in nerve excitation were studied using two main approaches. In one approach the nature of the interactions between excitable sites and the underlying cytoskeletal proteins was studied. Using intracellularly perfused squid giant axons, it was found that application of tetrodotoxin or saxitoxin to the nerve caused a release of cytoskeletal proteins into the perfusion solution. The other approach used embryonic chick skeletal muscle to study the development of the excitable sites in muscle. The excitable sites were studied using a number of neurotoxins known to bind to these sites, including saxitoxin, tetrodotoxin, scorpion toxin, veratridine and batrachotoxin.

Publications:

Baumgold, J., Terakawa, S., Iwasa, K., and Gainer, H.: Membrane-associated cytoskeletal proteins in squid giant axons. J. Neurochem. 36(2): 759-764, 1981.

Fink, D.J., Russell, J.T., Brownstein, M.J., Baumgold, J., and Gainer, H.: Multiple rate components of axonally transported proteins in the hypothalamo-neurohypophysial system of the rat. J. Neurobiol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01031-13 LNC

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Conversion of Phenylalanine to Tyrosine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Seymour Kaufman	Chief, Lab. of Neurochem.	LNC NIMH
OTHER:	Sheldon Milstien	Research Chemist	LNC NIMH
	Michael Parniak	Visiting Associate	LNC NIMH
	Harvey Wilgus	Sr. Staff Fellow	LNC NIMH
	Berenice Gitomer	Visiting Fellow	LNC NIMH
	Robert Phillips	Staff Fellow	LNC NIMH

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.58	5.08	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Phenylalanine hydroxylase can undergo a spontaneous, Mn^{2+}-sensitive activation that selectively increases its tetrahydrobiopterin-dependent activity. Modification of a single sulfhydryl group on phenylalanine hydroxylase also leads to a marked, selective increase in this activity. The native hydroxylase binds more than 1 molecule of phenylalanine per molecule of enzyme.

Project Description:

The objective of this research project is the detailed description of the complex enzyme system that catalyzes the conversion of phenylalanine to tyrosine. The specific goal is the analysis of the structure, mechanism of action, and modes of physiological regulation of the essential components of the hydroxylation system. These components include phenylalanine hydroxylase, dihydropteridine reductase, and tetrahydrobiopterin.

A related objective is to use this basic knowledge of the phenylalanine hydroxylase system to increase our understanding of the disease, phenylketonuria, (PKU), as well as its variants. Specifically, the goal is to devise methods of detection and therapy for these diseases, as well as to understand their pathogenesis.

We have found two new mechanisms by which phenylalanine hydroxylase can be activated. First, the alkylation of a single sulfhydryl group on the enzyme by N-ethylmaleimide leads to a 20 to 30-fold activation. As with most of the other modes of activation found in this laboratory (e.g., by phosphorylation, by interaction with phospholipids, by limited proteolysis), this one too is only expressed when the enzyme is assayed in the presence of the natural cofactor, tetrahydrobiopterin. This is the first activation of the enzyme by a well-defined chemical modification. We have also carried out phenylalanine binding studies with the enzyme and found that the unactivated enzyme binds 1.5 moles of phenylalanine per 50,000 Mr subunit, whereas the N-ethylmaleimide activated enzyme binds 1.0 mol of phenylalanine per 50,000 Mr subunit. These results provide the first direct evidence that there is more than 1 binding site per subunit for the native enzyme. In all probability, one of these sites is the catalytic site and the other is a regulatory site.

The second new type of activation of phenylalanine hydroxylase occurs spontaneously when crude hydroxylase preparations are dialyzed and then incubated at room temperature for about an hour. This activation, too, results in a selective 3-fold increase in the tetrahydrobiopterin-dependent activity. The activation can be partially reversed by incubation of the activated enzyme in the presence of Mn^{2+}. Interestingly, 2 different pure preparations of phenylalanine hydroxylase appear to have undergone spontaneous activation during the course of their purification. Consistent with this idea, pure phenylalanine hydroxylase when incubated under the same conditions as those that lead to activation of the crude enzyme, does not become further activated. Spontaneous activation appears to be synergistic with activation by phosphorylation.

Publications:

Parniak, M., Hasegawa, H., Wilgus, H., and Kaufman, S.: On the phosphate content of rat liver phenylalanine hydroxylase purified by hydrophobic chromatography. Biochem. Biophys. Res. Commun. 99: 707-714, 1981.

Parniak, M., and Kaufman, S.: Rat liver phenylalanine hydroxylase: Activation by sulfhydryl modification. J. Biol. Chem., in press.

Kaufman, S., Hasegawa, H., Wilgus, H., and Parniak, M.: The regulation of hepatic phenylalanine hydroxylase activity by phosphorylation and dephosphorylation. Cold Spring Harbor Symposium on Protein Phosphorylation, in press.

Matthews, R.G., and Kaufman, S.: Characterization of the dihydropterin reductase activity of pig liver methylenetetrahydrofolate reductase. J. Biol. Chem. 255: 6014-6017, 1980.

Nixon, J.C., Lee, C.-L., Milstien, S., Kaufman, S., and Bartholome, K.: Neopterin and biopterin levels in patients with atypical forms of phenylketonuria. J. Neurochem. 35: 898-904, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01032-13 LNC

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biosynthesis of Catecholamines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Seymour Kaufman	Chief, Lab. of Neurochem.	LNC NIMH
OTHER:	Gregory Kapatos	Staff Fellow	LNC NIMH
	Bruce Gomes	Staff Fellow	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.83	2.33	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The activity of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of catecholamines, is dependent upon a reduced unconjugated pteridine, tetrahydrobiopterin. We are actively pursuing enzyme purification procedures for tyrosine hydroxylase using as tissue sources both bovine striata and the adrenergic neuroblastoma, N1E115. Pteridine biosynthesis by rat brain, rat pineal gland, and adrenergic neuroblastoma, is being investigated in an attempt to elucidate the pathway by which pteridines are synthesized. The cellular mechanisms which regulate this synthesis, and the interactions between tyrosine hydroxylase and pteridines, are also being investigated.

Project Description:

Tyrosine Hydroxylase

Tyrosine hydroxylase from beef brain striata has been purified 23-fold from an extract of an acetone powder. If this enzyme preparation is treated with a cyclic AMP-dependent protein phosphorylation system, there is a change in the pH-dependence of the enzyme activity. The pH optimum at saturating tetrahydropterin concentration is shifted from below pH 6 to about pH 6.7 . At pH 7, activation is expressed mainly as an increase in Vmax, whereas at pH 6, activation is expressed mainly as a decrease in Km for the pterin cofactor. Furthermore, even with the control enzyme the Km for pterin cofactor declines precipitously as the pH is increased from 6 toward neutrality. Similar data were obtained with a G-25 Sephadex-treated rat striatal tyrosine hydroxylase. Experiments in which rat striatal synaptosomes were used demonstrated that the in situ activation of tyrosine hydroxylase by phosphorylating conditions is expressed primarily as an increase in the maximum rate of dopamine synthesis. These results indicate that changes in tyrosine hydroxylase activity caused by cyclic AMP-dependent protein phosphorylation will depend to a large extent on the pH of the tyrosine hydroxylase environment.

In contrast to the bovine enzyme preparation, tyrosine hydroxylase partially purified from neuroblastoma exhibits characteristics which suggest that some type of enzyme activation has occurred in situ. These characteristics are similar to those of the bovine or rat brain enzyme which has been activated in vitro, and they include pH optimum, Km for the reduced pterin cofactor, and an inability to be activated in vitro by protein phosphorylating conditions.

Pteridines

Hyperphenylalanemia in which neurological disorders persist despite dietary control of blood phenylalanine levels has been described. This condition is produced by defects in the phenylalanine hydroxylase system other than in phenylalanine hydroxylase itself, and is characterized by a deficiency of dihydropteridine reductase or a defect in the biopterin biosynthetic pathway leading to a deficiency of tetrahydrobiopterin. We have investigated the efficacy of pterin replacement therapy as a treatment for these disorders.

The content of tetrahydrobiopterin in rat brain was found to be doubled by peripherally administered tetrahydrobiopterin. Moreover, the natural l diastereoisomer of tetrahydrobiopterin was more effective than the unnatural d configuration in elevating brain tetrahydrobiopterin levels. The model pteridine, 6-methyltetrahydropterin was ten times more efficient than tetrahydrobiopterin in crossing the blood brain barrier. While no evidience for a specific uptake mechanism for concentrating 6-methyltetrahydropterin in cells containing tetrahydrobiopterin was detected, the pterin was found in its presumed site of action, the nerve ending. The clearance of 6-methyltetrahydropterin from blood was extremely rapid, displaying a t-1/2 of 3 hours. Replacement therapy with reduced pterins may therefore be effective in the treatment of the neurological disorders associated with the variant forms of hypephenylalanemia now known to result from defects in the biosynthesis or metabolism of tetrahydrobiopterin in the central nervous system. On a more general note, reduced pterin administration might provide a novel pharmacological technique for increasing the biosynthesis of catecholamines and serotonin in both the brain and periphery, and could therefore be useful in the treatment of disorders suspected to result from deficits in biogenic amine biosynthesis, such as Parkinsons disease and some forms of depression.

We are using cell-fee preparations from rat striata, rat pineal glands in organ culture, and an adrenergic neuroblastoma tissue culture system to investigate the biopterin biosynthetic pathway and the cellular processes which regulate this synthesis. This work, like that involving our studies of pterin replacement, finds its roots in the clinical literature.

We have described a cell-free preparation from the striatum of the rat which is capable of synthesizing biopterin from guanosine 5'-triphosphate. With the use of this preparation we have demonstrated that sepiapterin (6-lactyl-7,8-dihydropterin) is an intermediate in biopterin synthesis. Experiments performed with rat brain in vivo strongly suggest that 7,8-dihydrobiopterin synthesized from intraventricullarly administered sepiapterin, presumably by the action of sepiapterin reductase, is the precursor for 5,6,7,8-tetrahydrobiopterin, the functional cofactor for pterin dependent enzymes in rat brain.

Pineal glands in organ culture were found to synthesize and release biopterin and are able to maintain in vivo concentrations of biopterin for up to 54 hours in vitro. The intracellulaar biopterin content was reduced 50% by treatment with (l)-norepinephrine, cyclic AMP derivatives or phosphodiesterase inhibitors, but not by (d)-norepinephrine, indicating that biopterin levels are regulated by an adrenergic-cyclic AMP mechanism. The decline in tissue biopterin was produced mainly by an inhibition of biosynthesis, was maximal at 6 hours, and was not associated with either an increase in biopterin release or a shift in the reduction state of the biopterin. This is the first indication that the biosynthesis of biopterin within neural target tissue is under adrenergic control.

The adrenergic neuroblastoma N1E115 has been found to synthesize not only tetrahydrobiopterin but also 7,8-dihydro and possibly 5,6,7,8-tetrahydroneopterin. To our knowledge this tissue culture system is the only model available for studying pteridine biosynthesis in humans, which, like the neuroblastoma, synthesize neopterin. We have demonstrated that the synthesis of biopterin in this cell line can be inhibited by exogenously applied tetrahydrobiopterin, 7,8-dihydrobiopterin, and sepiapterin. These pteridines also inhibit the biosynthesis of neopterin. It would therefore appear that either all of these pteridines or their common cellular product, tetrahydrobiopterin, are capable of inhibiting biopterin biosynthesis. This may the the mechanism by which these cells maintain steady state levels of biopterin.

Publications:

Kapatos, G. and Kaufman, S.: Peripherally administered reduced pterins do enter the brain, Science 212: 955-956, 1981.

Kapatos, G., Kaufman, S., Weller, J.L., and Klein, D.C.: Adrenergic-cyclic AMP regulation of biopterin biosynthesis in the pineal gland. In Usdin, E. (Ed.): Second Conference on Structure and Function of Monoamine Enzymes. Macmillan Publishers Ltd., London, England, in press.

Kapatos, G., Katoh, S., and Kaufman, S.: Identification of 6-lactyl-7,8-dihydropterin (sepiapterin) as an intermediate in biopterin biosynthesis by a cell-free preparation from rat striatum. In Usdin, E. (Ed.): Second Conference on Structure and Function of Monoamine Enzymes. Macmillan Publishers Ltd., London, England, in press.

Kapatos, G., Kaufman, S., Weller, J.L., and Klein, D.C.: Development and regulation of biopterin in the pineal gland of the rat. In Klein, D.C. (Ed.): Symposium on the

Developmental Neurobiology of the Melatonin Generating System. Karger AG, Basel, Switzerland, in press.

Kapatos, G., Kaufman, S., Weller, J.L., and Klein, D.C.: Biosynthesis of biopterin: Adrenergic-cyclic AMP inhibition in the pineal gland. Science, in press.

Pollock, R.J., Kapatos, G., and Kaufman, S.: Effect of cyclic AMP-dependent protein phosphorylating conditions on the pH dependent activity of tyrosine hydroxylase from beef and rat striata. J. Neurochem. , in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01034-13 LNC

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Biochemical Basis of Skeletal Muscle Hypertrophy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Seymour Kaufman	Chief, Laboratory of Neurochemistry	LNC	NIMH
OTHER:	Herman Vandenburgh	Senior Staff Fellow	LNC	NIMH
	Rudolf Billeter	Visiting Fellow	LNC	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.08	2.08	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this project is the elucidation of the biochemical mechanism of compensatory skeletal muscle hypertrophy. These studies will give greater insight into the physiological regulation of normal and pathologic skeletal muscle metabolism and into the role of the nervous system in this regulation. Present topics of investigation include the relationship between stretch-induced hypertrophy and muscle protein synthesis, degradation, and changes in ion levels and phosphorylation of proteins.

Project Description:

One hypothesis for the mechanism of skeletal muscle hypertrophy is that it is due to mechanical stretching of the muscle which leads to increased tension and increased work. This hypothesis has been confirmed with the use of a technique developed in this laboratory of stretching monolayers of fowl embryonic skeletal myotubes differentiated in vitro. A continuous 10 percent stretch of the cells leads to increased amino acid accumulation, increased incorporation of amino acids into total cellular protein and muscle specific protein (myosin heavy chain), increased accumulation of total cellular protein and myosin heavy chain and decreased total cellular protein degradation. These changes are similar to those seen in in vivo models of skeletal muscle hypertrophy. Having established an in vitro model for skeletal muscle hypertrophy, we are currently testing several biochemical models for the stimulation of cell growth in this system. These models include changes in the activity of the sodium pump and in intracellular levels of cyclic nucleotides. Stretch of the skeletal myotubes has been found to activate the membrane Na pump (Na^+-K^+ ATPase). This Na pump activation appears to be essential for stretch-induced muscle cell growth since ouabain, a specific Na pump inhibitor, prevents the stretch-induced stimulation of amino acid transport and protein synthesis. Activation of the Na pump by stretch is due to an increase in the Vmax of the enzyme with little change in its Km. [^{3}H] ouabain binding studies indicate that there is no increase in the number of membrane Na pumps in response to stretch; therefore, the Vmax increase appears to be due to activation by stretch of the Na pumps already present in the cell membrane.

Futher analysis of the mechanism by which mechanical activity of the muscle cells stimulates amino acid transport indicates that the stimulation: a) does not require serum or any other growth promoting macromolecules in the medium; b) occurs by a mechanism similar to serum stimulation of amino acid transport since stretch has no effect on amino acid transport when transport is maximally stimulated by serum in the medium and c) is insensitive to the microfilament depolymerizer, vincristine. The involvement of transmembrane ion fluxes in this process is currently being tested.

Publications:

Vandenburgh, H., and Kaufman, S.: Protein degradation in embryonic skeletal muscle. Effect of medium, cell type, inhibitors, and passive stretch. J. Biol. Chem. 255: 5826-5833, 1980.

Vandenburgh, H.H., and Kaufman, S.: Regulation of skeletal muscle protein turnover by the plasma membrane Na pump. Acta IUPS 14, 1981, abstract.

Vandenburgh, H.H., and Kaufman, S.: Short and long term modification of skeletal muscle Na pump activity. Effects on muscle protein turnover. In Mechanism of Muscle Adaptation to Functional Requirements (F. Guba, G. Marechal, O. Takacs, Eds.), Akademiai Kiado, Publishing House of the Hungarian Academy of Science, Budapest, in press (1980).

Vandenburgh, H.H., and Kaufman, S.: Stretch-induced growth of skeletal myotubes correlates with activation of the Na pump. J. Cell Physiol. 1981, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01035-13 LNC

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Process of Lysogeny

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard Nash	Medical Officer (Research)	LNC	NIMH
Other:	Thomas Pollock	Guest Worker	LNC	NIMH
	Brenda Lange-Gustafson	Guest Worker	LNC	NIMH
	Nancy Craig	Guest Worker	LNC	NIMH
	Carol Robertson	Biologist	LNC	NIMH

COOPERATING UNITS (if any) Division of Biology and Medicine, Brown University; Laboratory of Molecular Genetics, NICHD; Laboratory of Molecular Biology, NCI; Laboratory of Molecular Biology, NIAMDD

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.7	3.7	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An E. coli protein required for integration of bacteriophage lambda DNA into the host chromosome has been purified and characterized as to subunit structure and genetic makeup. Footprinting experiments reveal that this protein binds specifically to the viral recombining site and may stabilize the interaction of a viral protein, Int, that also must interact with this site. A variant Int protein that may have an altered affinity for or dependence on the host protein has been purified; characterization of its interaction with the components of the recombination system is underway. Indirect evidence, based on the formation of knotted DNA during recombination, indicates that recombination proteins and the DNA of one of the recombining sites may be organized into a structure like that of the nucleosome subunits of chromatin.

Project Description:

Lysogeny is the outcome of a bacterial virus infection in which the virus persists indefinitely within the host bacterium. In the case of bacteriophage lambda, this stable relationship is achieved through the integration of the viral DNA into the host genome, permitting the virus to be replicated passively in synchrony with the E. coli chromosome. This laboratory has been studying the biochemical mechanism that leads to the union of the viral and host DNA. After successfully demonstrating this reaction in a cell-free system, we investigated the nature of the substrates, products, enzymes and cofactors that are involved in this reaction. We discovered that supercoiled DNA is the primary substrate and that the products of recombination of such DNA imply a mechanism for recombination that is very conservative. Among the features of the parental DNAs that are conserved during integrative recombination are the nucleotides around the point of breakage and rejoining, the chemical energy of the phosphodiester backbone, and at least 85% of the supercoils. We have also identified and purified the proteins involved in integrative recombination. A viral protein, Int, appears to be directly responsible for the breakage and reunion event. Int protein binds to the two parental DNAs precisely at the spot where recombination takes place. Moreover, purified Int protein contains a topoisomerase activity, i.e. can reversibly break and reseal the strands of DNA. However, Int cannot achieve recombination by itself. It must be supplemented by a second protein, IHF (for Integration Host Factor), that is produced by the bacterial host.

We have recently purified IHF to near homogeneity. Under nondenaturing conditions, IHF behaves as a single species of molecular weight 20,000; gel electrophoresis under denaturing conditions reveals two polypeptides of molecular weight 11,000 and 9,500. The conclusion that IHF is a complex of two polypeptides is supported by genetic experiments. Mutations in two widely separated genes, himA and hip, affect IHF function. Indeed, we have shown that the 11,000 molecular weight subunit of IHF is the product of the himA gene. Since extracts of himA$^-$ cells are complemented by extracts of hip$^-$ cells to produce active IHF, we presume that the 9,000 molecular weight subunit of IHF is encoded or controlled by the hip gene. Our current studies focus on the role of IHF in recombination. The purified protein lacks any trace of endonuclease or topoisomerase activity, supporting the contention that Int, not IHF, carries the strand exchange functions for recombination. We have recently asked whether IHF interacts specifically with DNA. We employed the footprinting technique of Galas and Schmitz, which assays the ability of a protein to protect a stretch of nucleotide sequence from digestion by nucleases. IHF was found to specifically protect three regions of the viral recombining site (attP). Each protected region is 25 - 30 base pairs long and to a rough approximation is positioned adjacent to a segment of DNA shown previously to be complexed with Int protein. Preliminary studies indicate that IHF bound to DNA stabilizes the interaction of Int with the adjacent DNA; this may be the primary function for IHF in integrative recombination. If this were the case, one would expect to be able to isolate tight binding variants of Int that have a decreased requirement for IHF. We are studying such a mutant protein, the product of the int-h allele. This variant was isolated by our colleagues at the University of Michigan as mutant virus that could achieve lysogeny of bacteria mutant for IHF. We have cloned the int gene from this virus in a plasmid vector, and have partially purified the resultant Int-h protein. The purified protein displays altered behavior in several in vitro assays; e.g. recombination with mutant IHF and recombination of non-supercoiled DNA. We plan to determine whether these properties reflect an altered affinity of Int-h for DNA or an altered interaction of Int-h with IHF.

Recent experiments have uncovered a surprising consequence of the interaction of recombination proteins with attP. These studies used as the recombination substrate circular DNA molecules containing both attP and the bacterial recombining site (attB) in an orientation such that recombination between the attachment sites leads to inversion of the intervening segment of the substrate circle. The major finding is that the recombinant circles are frequently knotted, even when the substrates are prepared so that the recombining sites are located only 300 base pairs apart on a circle without supercoils. Models for recombination that evoke a simple juxtaposition of attachment sites followed by a cycle of breakage and reunion do not explain this result. We deduce that at least one site is organized into a nucleosome-like structure prior to synapsis. This has the effect of entangling the DNA in a limited way such that the observed proportion of knotted and unknotted recombinants follows naturally from juxtaposition and strand exchange. We think it likely that the nucleosome-like structure is a consequence of Int and IHF binding to attP and that this structure prepares attP for synapsis with attB.

Publications:

Mizuuchi, K., Gellert, M., Weisberg, R. A. and Nash, H. A.: Catenation and supercoiling in the products of in vitro lambda integrative recombination. J. Mol. Biol. 141: 485-494, 1980.

Pollock, T. J. and Nash, H. A.: Catenation of The Products of Integrative Recombination: Comparison of Supertwisted and Nonsupertwisted Substrates. In Alberts, B. and Fox, D. F. (Eds.): Mechanistic Studies of DNA Replication and Genetic Recombination - ICN-UCLA Symposia on Molecular and Cellular Biology, Volume XIX. New York, Academic Press, 1980, 953-961.

Nash, H. A., Mizuuchi, K., Enquist, L., and Weisberg, R. A. Strand exchange in lambda site specific recombination: Genetics, biochemistry and models. In Cold Spring Harbor Symposia on Quantitative Biology 45: 417-428, 1981.

Miller, H. I., and Nash, H. A. Direct role of the himA gene product in phage lambda integration. Nature 290: 523-526, 1981.

Nash, H. A., Site-Specific Recombination Protein of Phage Lambda, The Enzymes 14, in press, 1981.

Nash, H. A. and Robertson, C. A., Purification and Properties of the E. coli Protein Factor Required for Lambda Recombination. J. Biol. Chem., 1981, in press.

Nash, H. A.: Integration and Excision of Bacteriophage Lambda: The Mechanism of Conservative Site Specific Recombination. Ann. Rev. Genet. 15, 1981, in press.

Honors and Awards:

ADAMHA Administrator's Award for Meritorious Achievement, 1981

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01037-13 LNC

PERIOD COVERED

October 1, 1980 through September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Role of the Cell Membrane in Cellular Organization, A Molecular Study.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David M. Neville, Jr.	Chief, Section on Biophys. Chem.	LNC	NIMH
OTHER:	Richard Youle	Staff Fellow	LNC	NIMH
	Steven Esworthy	Staff Fellow	LNC	NIMH
	Ateeq Ahmad	Visiting Associate	LNC	NIMH

COOPERATING UNITS (if any)

Genetics and Biochemistry Branch, NIAMDD

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Biophysical Chemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

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SUMMARY OF WORK (200 words or less - underline keywords)

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. Specifically we have studied one type of interaction, plasma membrane receptor mediated entry of proteins into the cell cytosol. These studies have been done by developing techniques to construct artificial protein hybrids containing the active fragment of a toxin and another receptor specific binding protein. Such artificial protein hybrids may have value as a new class of pharmacologic reagents.

Project Description:

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. The major specific aim of the program is to synthesize a new class of pharmacologic reagents which permit the modulation of specific cell types in a manner unobtainable with conventional reagents. This is being accomplished by the synthesis of artificial protein hybrids which utilize the process of receptor-mediated protein transport.

A wide variety of proteins are capable of entering cells by receptor-mediated transport processes. Having gained entry these proteins are directed to specific cellular compartments where they exert either a physiological or pathological function.

In general it appears that only a discrete portion of these proteins contain the receptor binding activity which is involved in the entry process while another portion of the protein performs the intracellular function. Therefore, it is possible to split and reassemble two such proteins with a new combination of receptor entry specificity and intracellular function. Such proteins we call artificial hybrid proteins, and previous reports from this laboratory have suggested that such hybrids should have utility both as probes of entry processes and as a new class of pharmacologic reagents with tailor made receptor and therefore cell type specificity.

Clinical Applications:

A hypothetical example of the clinical utility of an antificial hybrid protein reagent is the following. Many malignant tumors are known to carry tumor specific receptors on their cell surfaces (tumor specific antigens). An artificial hybrid protein containing a functional group binding to these specific receptors and linked to a highly toxic intracellular functional group (diphtheria toxin fragment A) might function as a tumor specific toxic reagent. The advantage of this type of reagent over currently available antitumor drugs would be the property of cell type specificity.

Artificial hybrid protein technology also holds the promise for constructing new types of neuropharmacologic reagents. Tetanus and botulinum toxins inhibit the release of inhibitory and excitatory neurotransmitters respectively, by virtue of their intracellular functional A chains. By linking these A chains to proteins which bind to only certain areas of the brain, a high degree of regional specificity might be achieved, and it is likely that the regional specificity would be mirrored by behavioral specificity.

Major Findings:

Previously we reported the construction of two different cell type specific toxic reagents by modifying the receptor specificity of the toxin ricin by covalently coupling either mannose-6-phosphate (Man-6-P) or anti-Thy 1 monoclonal antibody. In the presence of lactose which blocks the usual ricin entry route these hybrid toxins enter cells via the alternate receptors, Man-6-P or Thy 1, and specifically kill cells carrying these receptors.

We now have shown that the mechanism of efficient hybrid toxin entry into the cytosol compartment involves an intracellular interaction with a cellular component which binds to the B chain of the ricin at its galactose binding site. This interaction is independent of cell surface binding which in these cases only occurs at Man-6-P or Thy 1 receptors. This finding was elucidated by performing a site specific modification of one or

two of the ricin tyrosine residues at the galactose binding site using N-acetylimidazole. The resulting hybrids made with O-acetyl ricin were diminished in activity by 90% although their binding to cell surface receptors was unchanged.

Further evidence supporting the hypothesis of a common intracellular step in the toxicity of ricin and hybrid ricin was obtained by the selection of ricin transport minus mutants using Man-6-P ricin plus lactose under conditions where all binding was via the Man-6-P receptor.

These findings have profound implications for the construction of efficient hybrids. The most useful reagents must have the ricin B chain galactose site blocked in the extracellular compartment (to prevent non-specific entry via the ricin route) but this site must be functional inside the cell. This poses an interesting challenge for future work.

Publications:

Youle, R.J., Murray, G.J. and Neville, D.M., Jr.: Studies on the galactose-binding site of ricin and the hybrid toxin Man-6-P-ricin. Cell 23: 551-559, 1981.

Heagy, W. and Neville, D.M., Jr.: Kinetics of protein synthesis inactivation by diphtheria toxin in toxin resistant L cells: Evidence for a low efficiency receptor-mediated transport system. J. Biol. Chem. in press.

Robbins, A.R., Myerowitz, R., Youle, R.J., Murray, G.J. and Neville, D.M., Jr.: The mannose-6-phosphate receptor of Chinese hamster ovary cells. Isolation of mutants with altered receptors. J. Biol. Chem. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01081-11 LNP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The Role of Sensorimotor Cortex in Control of Voluntary Movement

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. V. Evarts	Chief	LNP	NIMH
	R. Nelson	Staff Fellow	LNP	NIMH
	C. Fromm	Guest Worker	LNP	NIMH
	J. Kröller	Guest Worker	LNP	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Neurophysiology

SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project involves utilization of single neuron recording and operant conditioning techniques in behaving monkeys to study brain mechanisms underlying voluntary movement. Monkeys are trained to make precise movements of a handle whose movement controls a visual display, and stimuli are delivered via the handle by means of an electronically controlled torque motor in order to determine how sensory feedback is processed. Using these methods we have obtained the first data on activity of identified descending output neurons from somatosensory cortex during voluntary movement in the monkey and have found that these neurons have three properties in common with the descending output neurons of motor cortex: (i) they exhibit discharge prior to movement onset, (ii) their discharge frequency varies as a function of strength of muscular contraction and (iii) they show reflex responses to afferent stimuli occurring during movement. These findings suggest that in addition to its widely recognized role in perception, somatosensory cortex also has a role in control of movement.

Project Description:

In the previous work of this project on motor cortex, primary attention has been focused on a class of motor cortex neurons which send their axons to the spinal cord via the pyramidal tract. These neurons are referred to as pyramidal tract neurons (PTNs) and are of particular importance because they convey the final output from motor cortex to the spinal cord regions from which impulses pass out to the muscles to generate movement. In recent experiments, we have devoted particular attention to PTNs located in the sensory receiving area of the cerebral cortex. The purpose of these new observations was to provide further knowledge of the functional differentiation between motor cortex (MI) and sensory cortex (SI). Unlike spinal cord output neurons to muscle, which originate entirely in the motor part of the cord, cerebral cortex output neurons to spinal cord are located in both MI and SI. Our experimental paradigm involved comparing MI and SI PTNs with respect to (i) temporal relations to onset of movement (ii) discharge frequencies as a function of strength of muscular contraction and (iii) responsiveness to afferent stimuli.

Major Findings:

I. SI PTN Discharge prior to Movement Onset.

We compared timing of 208 PTNs in MI and 72 PTNs in SI in a paradigm in which a monkey was rewarded for (i) holding a handle in one of three different angular positions (15^{o} supinated, 0^{o} vertical, and 15^{o} pronated position) on the basis of a visual display and (ii) positioning the handle between these three zones by pronation or supination movements whenever a shift of the visual display indicated when and where to move. The electromyogram (EMG) of muscles involved in this task was recorded so as to relate the timing of EMG changes to the timing of changes of PTN activity.

Unlike the cytoarchitectonically homogeneous MI (area 4), SI contains several subdivisions: from anterior to posterior within SI are areas 3a, 3b, 1 and 2. These four SI areas differ with respect to the somatosensory submodality that they receive. Immediately behind area 2 is area 5, a somatosensory association zone, and PTNs are located here as well as in the four subdivisions of SI. In view of the known differences between these areas, results for PTNs have been subdivided according to the cortical areas in which they were located. For area 3a of SI, all PTNs, but none of the non-PTNs, discharged before the earliest EMG changes of any arm muscle. Discharge before the earliest EMG onset occurred less frequently in other SI areas, with such prior discharge occurring in 55% of PTNs recorded in an area 2/5 junctional region and in 20% of the PTN population in areas 2 and 1. There were no significant timing differences between PTNs and non-PTNs in these SI regions. For MI (area 4), 80% of the PTNs discharged in advance of EMG onset. The total PTN population of MI lagged that of area 3a PTNs by an average of 23 ms ($p < .025$); this timing difference became greater (48 ms) and highly significant ($p < .001$) if we compared mean onset time of area 3a PTNs with that of a subgroup of 33 PTNs in area 4 which were located in the rostral bank of central sulcus adjacent to area 3a.

II. SI PTN Discharge Varies with Steady-State Load.

SI PTNs change discharge frequency both with different external loads (position held constant), and with different joint positions (external load held constant). This corresponds to changes in level of muscle activity since an increase in

muscle activity with reduced muscle length and with constant external load results from the length-tension properties of muscle; in parallel with these muscle changes many SI PTNs were more active with loads requiring more muscle activity and these PTNs also exhibited higher levels of maintained tonic discharge for the corresponding joint position.

III. SI Responses to Afferent Input.

The data that have now been summarized show that motor and sensory cortex PTNs have two features in common: discharge prior to muscular contraction and changes in discharge frequency as a function of strength of muscular contraction. The third feature according to which MI and SI PTNs were compared was responsiveness to afferent input. Two types of stimuli were used to elicit PTN activity. The first type was a brief unpredictable halt of voluntary movement brought about by electronically "clamping" the servo-motor just as the movement was starting. The latencies of responses to afferent input were shortest in non-PTNs of area 3a (mean: 12 ms) and were significantly longer in area 3a PTNs (mean: 20 ms). There were no clear latency differences between different SI PTN populations, but the group mean of 22 ms for their afferent responses was significantly shorter than the mean of 26 ms in MI PTNs. Across all cortical subdivisions there was a significant tendency for larger rapidly conducting PTNs to show dynamic ramp responses, while dynamic-static and purely static responses prevailed in slow, smaller PTNs.

Interpretations and Conclusions.

Prior studies of motor cortex PTNs during voluntary movement have shown that these neurons exhibit centrally programmed discharge prior to movement onset and that, in addition, they show reflex responses to peripheral stimuli. These PTN reflex responses (called long-loop reflexes to contrast them with the analogous short-loop spinal cord reflexes) provide for automatic servo-control of centrally programmed PTN output during voluntary movement. The present results show that long-loop reflexes and centrally programmed discharge prior to movement are also present in many sensory cortex PTNs and that these PTNs can act on their target neurons in advance of movement. The results on PTNs in area 3a, the subdivision of SI that receives inputs from muscle afferents were of particular interest, for within this area the PTNs discharged prior to movement onset but the non-PTNs failed to do so. While lagging PTNs in onset of activity with voluntary movement, area 3a non-PTNs were earlier than PTNs in responsiveness to peripheral stimuli. Unfortunately, we do not have information as to the laminar location of 3a non-PTNs, but it is tempting to propose that they are close to the input of the cortical column. Though delayed by an average of 8 ms with respect to non-PTNs, the reflex responses of PTNs in area 3a (and in the other SI areas) were nevertheless earlier than the reflex responses of motor cortex PTNs. The existence of these SI PTN reflex outputs to brainstem and spinal cord means that quite aside from further cortico-cortical processing of SI activity leading to perception, conscious awareness and decision-making, there are immediate SI reflex outputs that must play a part in continuous regulation of the excitability of lower centers during movement.

Proposed Course:

Within the next year we intend to extend these studies to obtain more data on PTNs and non-PTNs in sensorimotor cortex.

Significance to Biomedical Research and to the Program of the Institute:

This project seeks to apply basic research results on central control of voluntary movement to an understanding of normal and abnormal movements in man. To the extent that the project succeeds, it will contribute to the research goals of the NIMH. Thus far, the project has shown that the laws of reflex action, long known to operate at the level of the spinal cord motoneuron, also operate at the level of the cerebral cortex in the course of volitional movements. Motor cortex neurons are impinged upon by afferent inputs which constitute the incoming limb of a transcortical servo loop. Thus, the phylogenetically new motor cortex of the mammal is subject to the same laws of reflex action that characterize phylogenetically older components of motor control systems. But in addition to being driven by a servo system which stabilizes movement and posture, motor cortex can be driven by a second major set of inputs, and it is this second set of inputs that underlies internally generated motor programs. These programs, reaching the motor cortex from the thalamus, are themselves a product of activity in red nucleus, basal ganglia, and cerebellum.

Publications:

Evarts, E.V.: Brain Mechanisms in voluntary movement. In: Neural Mechanisms in Behavior: A Texas Symposium, D. McFadden (ed.), Springer-Verlag, New York, 1980 (pp. 223-259).

Evarts, E.V.: Moving. In: Behavioral Neuroscience, An Introduction, C. W. Cotman and J. L. Mcgaugh, Academic Press, New York, 1980 (pp. 461-513).

Evarts, E.V.: Functional Studies of the Motor Cortex. In: The Cerebral Cortex: Proceedings of a Neurosciences Research Program Colloquium, F. O. Smith, F.G. Worden and S.G. Dennis (eds.). Karger, Basel, 1980 (pp. 263-283).

Evarts, E.V.: Closing Remarks on the Neural Mechanisms of Voluntary Movements and Precentral Motor Area. In: Regulatory functions of the CNS, Motion and Organization Principles, J. Szentágothai, M. Palkovits, J. Hámori (eds.). Adv. Physiol. Sci., vol. 1, 1980, (pp. 131-140) Presented at the 28th Internatl. Cong. of Physiol. Sciences.

Evarts, E.V. and Fromm, C.: Information processing in the Sensorimotor Cortex during voluntary movement. In: Motivation, Motor and Sensory Processes of the Brain, Progress in Brain Research, vol. 54, 1980 (pp. 143-155).

Evarts, E.V.: Sherrington's Concept of Proprioception. Trends Neurosci., 4: 44-46, 1981.

Fromm, C., and Evarts, E. V.: Relation of size and activity of motor cortex pyramidal tract neurons during skilled movements in the monkey. J. Neurosci., 1: 453-460, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01084-10 LNP

PERIOD COVERED
October 1, 1980 to July 31, 1981

TITLE OF PROJECT (80 characters or less)

Eye-Head Coordination in the Primate: Neural Substrate and Plasticity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. G. Lisberger	Staff Fellow	LNP NIMH
Other:	F. A. Miles	Research Physiologist	LSR NEI
	D. S. Zee	Asst. Professor	Johns Hopkins Univ.

COOPERATING UNITS (if any)
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Department of Neurology & Ophthalmology, Johns Hopkins University

LAB/BRANCH
Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-range aim of this project is to determine the neural basis of adaptive mechanisms responsible for establishing and maintaining appropriate performance levels in the oculomotor system of the rhesus monkey. The mechanism currently under study relates to the gain of the vestibulo-ocular reflex. One series of experiments showed that changes in the gain of the vestibulo-ocular reflex are "frequency selective"; adaptation during passive sinusoidal oscillation at one temporal frequency produced large changes in gain at that frequency and smaller changes at adjacent frequencies. A second series of experiments quantified the serious effects of bilateral flocculectomy on the adaptive mechanism and on the optokinetic response. A third series of experiments showed that adaptation of the vestibulo-ocular reflex causes some changes in the twitch of eye velocity evoked by single electrical shocks applied to the vestibular nerve; the changes occurred in parts of the twitch that can be attributed to multisynaptic vestibular pathways to extraocular motoneurons.

Objectives:

Our continuing objective has been an understanding of the adaptive mechanisms responsible for establishing and maintaining appropriate performance levels in the oculomotor system. In the past year we have focused on one mechanism: the regulation of gain in the vestibulo-ocular reflex of rhesus monkeys. One series of experiments produced adaptation during passive, sinusoidal vestibular oscillation at one frequency, and measured the effect on the gain and phase shift of the vestibulo-ocular reflex at adjacent frequencies. A second series of experiments was undertaken to extend our previous observations concerning the effect of bilateral ablation of the cerebellar flocculus on the adaptive process. A third series was designed to evaluate the site of neural modifiability by studying the eye velocity twitch evoked by single electrical shocks to the vestibular nerve in normal and adapted monkeys.

Methods Employed:

The adaptive control of the gain of the vestibulo-ocular reflex was studied using previously described techniques. Briefly, the gain of the vestibulo-ocular reflex was increased by prolonged exposure to x2-telescopic spectacles or decreased by exposure to goggles providing a visual field that was fixed with respect to the head. In addition to the previous strategy of allowing the monkey to undergo adaptation during free and active head turns, we have devised a new procedure for adaptation during passive vestibular stimulation. The monkey was fitted with spectacles and placed in a primate restraining chair to which his head was fixed. He was subjected to continuous passive oscillation in the light at one selected sinusoidal frequency. Eye movement was monitored continuously on a chart recorder and the paper was scored to estimate the number of minutes of spectacle experience (i.e. monkey awake with eyes open) actually endured. At regular intervals, the spectacles were removed, the lights extinguished and the vestibulo-ocular reflex was tested over a wide range of sinusoidal frequencies in complete darkness.

Bilateral flocculectomies were performed by Dr. David S. Zee at Johns Hopkins University on four monkeys using suction ablation under direct visualization.

Horizontal optokinetic responses were elicited by seating monkeys inside a full-field striped cylinder that rotated at constant velocity; the resulting nystagmus consists of smooth tracking eye movements in the direction of stripe movement, interrupted at intervals by resetting saccades. After sudden illumination of the moving stripes, the velocity of the tracking eye movements shows an initial rapid rise (lasting a few hundred milliseconds) followed by a more gradual increase (lasting 10-15 sec); these rapid and gradual events are believed to represent two separate components of the optokinetic response. After the illumination goes out, there is an initial rapid decrease in smooth eye velocity followed by a long-lasting afterresponse (so-called optokinetic afternystagmus), the initial velocity of which is assumed to be an index of the level achieved by the gradual component of the prior optokinetic response.

A new technique was devised to allow electrical stimulation of both vestibular nerves in alert animals. Under surgical anesthesia, small holes were made in the calvarium, at stereotaxic locations appropriate for reaching the vestibular nerves. The following day, the monkey was placed in the usual experimental situation and lightly anesthetized so that bipolar stimulating electrodes could be introduced through the dura into the brain. The monkey was then allowed to recover from anesthesia so that the position of the electrodes could be monitored and finalized

by recording the eye movements evoked by electrical stimulation as the electrodes were advanced. When the eye movements indicated that the electrodes were on or very near the vestibular nerve, the electrodes were cemented in place and the monkey returned to his home cage. Application of single electrical stimuli to one nerve in the alert monkey produces a small twitch of eye velocity that is initially in the direction contralateral to the stimulated nerve, but that has a rebound in the direction ipsilateral to the stimulated nerve. Whereas the initial, contralateral response is probably due to activation of disynaptic and multisynaptic vestibular pathways to the extraocular motoneurons, the later, ipsilateral response is believed to be related in a complicated way to the gain of transmission through the multisynaptic pathways.

Major Findings:

1. Passive oscillation with spectacles produced larger changes in VOR gain at the adapting frequency than at adjacent frequencies. For example, adaptation at 0.2 Hz produced larger changes in VOR gain when tested at 0.2 Hz (average for the 4 monkeys reaching high gains of 1.66 and low gains of 0.42) than when tested at 2.0 Hz (up to 1.35 and down to 0.63). Similarly, adaptation at 2.0 Hz produced larger changes in VOR gain at 2.0 Hz (up to 1.62 and down to 0.39) than at 0.2 Hz (up to 1.10 and down to 0.62). While eye velocity remained 180 deg out of phase with head velocity at the frequency of adaptation, consistent small changes in phase shift were seen at adjacent frequencies. At frequencies above that used for adaptation, increases in VOR gain caused eye velocity to lag a perfect VOR while decreases caused eye velocity to show phase lead. Conversely, at frequencies below that used for adaptation, increases in VOR gain caused eye velocity to lead a perfect VOR while decreases caused phase lag. These observations suggest the existence of temporal frequency channels in the vestibulo-ocular reflex pathways.

2. Bilateral flocculectomy has profound effects on the adaptive capability in the vestibulo-ocular reflex, and on one component of the optokinetic response.

(i) Complete flocculectomy causes a virtually complete loss of VOR adaptation; even hours of passive oscillation at 0.2 or 2.0 Hz with x2- or x0-spectacles caused no change in the gain of the vestibulo-ocular reflex. Partial flocculectomy allows the retention of some adaptive capability. However, changes occur 5 to 10 times more slowly and attain an asymptotic level representing less complete adaptation than is found in the normal monkey.

(ii) After flocculectomy, the rapid component of the optokinetic response was considerably attenuated (gain, measured as the maximum eye velocity in the first 1 sec. divided by retinal slip, was only 50% of normal). However, the gradual component achieved a nearly normal amplitude and did so over a nearly normal time course.

3. Adaptation of the vestibulo-ocular reflex leads to consistent changes in some aspects of the eye velocity response to single electrical shocks applied to the vestibular nerve. The most reproducible effects were found in the amplitude of the later, ipsilateral rebound part of the twitch, which varied inversely in relation to the gain of the vestibulo-ocular reflex. Thus, increases in the gain of the vestibulo-ocular reflex caused the rebound to become negligibly small, while decreases in the gain of the vestibulo-ocular reflex caused the amplitude of the rebound to be slightly larger than normal. Although further experiments must be carried out to understand the implications of this finding, it is consistent with the suggestion that the site of the adaptable elements is the multisynaptic

vestibular pathways to extraocular motoneurons.

Significance to Biomedical Research and the Program of the Institute:

A great deal is known about the anatomy and physiology of eye movements. The discrete, rather machine-like quality of the oculomotor system has proved particularly amenable to the analysis of information processing at the level of the system and the single neuron. The fact that the system also displays plasticity opens up new approaches to the subject of motor learning at the cellular level in the primate central nervous system. Advances toward understanding the site and mechanism of motor learning in the oculomotor system represent important steps toward understanding the maturation of the normal nervous system and the self-repairing abilities of the diseased nervous system. The oculomotor deficits that follow lesions of the flocculus in the monkey demonstrate the necessity for the functional integrity of this region of the cerebellum for normal visual following and for adaptive regulation in the vestibular system. These findings also suggest further tests that might aid localization of the pathology in human patients with classical cerebellar signs.

Proposed Course:

On July 31, 1981, S. G. Lisberger will be leaving the NIH and this project will be terminated.

Publications:

Miles, F. A., and Lisberger, S. G. Plasticity in the vestibulo-ocular reflex: A new hypothesis. Ann. Rev. Neurosci., 4: 273-299. 1981

Lisberger, S.G., Miles, F. A., Optican, L. M., and Eighmy, B. B. Optokinetic response in monkey: underlying mechanisms and their sensitivity to long-term adaptive changes in vestibuloocular reflex. J. Neurophysiol., 45: 869-890, 1981.

Lisberger, S. G., Evinger, C., Johanson, G. W., and Fuchs, A. F. Relationship between eye acceleration and retinal image velocity during foveal smooth pursuit in man and monkey. In Press, to be published in August by J. Neurophysiol.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01090-05 LNP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Central Nervous System Functional Anatomy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Miles Herkenham	Research Psychologist	LNP	NIMH
Other:	Sandra Moon Edley	Staff Fellow	LNP	NIMH
	Ronald P. Hammer Jr.	Staff Fellow	LNP	NIMH
	Candace Pert	Pharmacologist	BP	NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.75	2.75	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A sensitive method for visualization of brain receptors by in vitro autoradiography featuring intact cell morphology was developed previously in this laboratory. By this method we have mapped the localization of opiate receptors in the brains of rats and other mammalian and non-mammalian vertebrates. Comparisons of tritiated naloxone binding with tritiated enkephalin binding have reinforced the notion of opiate receptor subtypes. These have been followed ontogenetically and phylogenetically and have been related to analogous receptor subtypes in the dopamine system. Applications are being pursued for the study of receptors and biologically active peptides and enzymes in unfixed brains of humans obtained at autopsy. The unfixed, cryostat-cut tissue is amenable for concurrent study of metabolic and functional mapping by 2-deoxy-D-glucose. One project underway will localize and compare phencyclidine (PCP) receptors in the brain with the altered brain metabolic activity produced by phencyclidine "anesthesia". The technique is used also for studies related to sleep, circadian rhythms and drug-induced sedation.

I. Studies of CNS Functional Neuroanatomy. Neurochemical Investigations.

Objectives:

Since the autumn of 1978 an extensive collaboration has been undertaken with Dr. Candace Pert in the Biological Psychiatry Branch of the NIMH. Our efforts have been aimed at developing new methods to localize specific brain regions, the sites of action of pharmacologically active drugs, and putative neurotransmitters. The discovery by Dr. Pert and others in 1973 of brain receptors for opiates and opioid peptides, and the subsequent localization of these receptors by neuroanatomical techniques, has opened up an important new area of anatomy. The task of finding the neuronal circuitry that is "plugged into" these receptor sites requires knowing precisely both the distribution of the receptors and terminal distributions of fiber pathways in any given region. This search for anatomical correlates is augmented by the use of immunocytochemical mapping techniques for selectively marking cells and axons containing the putative endogenous transmitters that are released onto the receptors. The assignment of a neurotransmitter to each of the identified CNS pathways is the ultimate goal of this type of effort.

Methods Employed:

We have successfully developed an *in vitro* autoradiographic technique for visualizing drug and neurotransmitter receptors in slide-mounted tissue slices. Fresh cryostat-cut brain slices are securely attached to glass slides by a process of thaw-mounting and subsequent drying at cold temperatures. Slides are then incubated in solutions containing a radiolabeled ligand. Excess and nonspecifically bound ligand is washed off in cold buffered rinses, and the slides are blown dry. The sections are fixed in hot paraformaldehyde vapors under a vacuum, defatted in xylene and alcohol rinses, dried and then dipped in radioactive-sensitive emulsion for autoradiography. Alternatively, fixed sections can be placed in an x-ray cassette and overlain with LKB tritium-sensitive film. The developed LKB autoradiogram then can be analyzed by a densitometer for quantification of receptor densities. While emulsion coated sections provide high resolution analysis through the microscope, LKB films can be computer-analyzed and the densities color-coded for instant visualization of densities.

Major Findings:

To our delight we found that paraformaldehyde vapors successfully "fixed" the tritiated ligand to the receptor as well as fixing the tissue itself. This technical breakthrough permits the use of traditional "wet" autoradiographic procedures for dipping slides into liquid nuclear track emulsion for the subsequent visualization or receptor localization. The combination of the *in vitro* labeling technique and "wet" autoradiography has resulted in opiate receptor localization whose resolution surpasses all previously published techniques. Through careful histological procedures we have also produced tissue of higher quality than previously attained. The same tissue is amenable to tract-tracing studies and enzyme histochemistry so that "double- and triple-labeling" experiments have been performed on alternate sections from the same animal. A published paper in press describes our first efforts at surmising "opiatergic" tracts from the observed concordances between the autoradiographic visualization of tract terminations and receptor distributions in corresponding brain loci. Another paper describes a perfect mosaic fit between opiate receptors, acetylcholinesterase staining and thalamic projections to the striatum in the rat. A major biochemical application was appreciated when we

discovered that these tissue sections can be used in place of brain homogenates to do routine studies of binding kinetics, saturation curves, structure-activity relationships, and so on. From this effort has come a major insight into the interconvertible nature of opiate receptor subtypes. Previous work in the literature has suggested distinctly different alkaloid or "mu" binding sites for morphine, naloxone, etc. and peptide or "delta" binding sites preferring enkephalin or its analogs. We have shown that the autoradiographic pattern of the peptide binding can be made to resemble that of alkaloid binding by adding ions, GTP and divalent metals to the incubation medium, suggesting that the opiate receptor subtypes may be different conformations of the same receptor molecule.

A consequence of this has been our re-classification of the opiate receptor subtypes based on their autoradiographic distribution patterns under various incubation conditions. Obviously, this had been an impossible outcome with analysis of homogenized tissue.

The opiate receptor in its various forms has been followed in ontogeny and phylogeny. We found the first indications that opiate receptors play a crucial role in normal development of rat forebrain. They appear very early in striatal development and, through a process of receptor elimination, are "sculpted" to form the intricate patterns that characterize the adult. These changes may influence the patterns of connections that are subsequently established. In evolution another startling observation has been made: the ratio of opiate receptor subtypes calculated for several vertebrates and one invertebrate is a reliable indicator of species relatedness and may mark an evolutionary trend towards greater receptor complexity.

The method has applicability for all receptor types for which a drug or transmitter ligand has been identified. In addition, it can be used for cells grown in culture and on non-neural body tissues. Perhaps more exciting is the fact that it can be used on human post-mortem tissue and may open new doors into receptor-related disease states.

II. Metabolic Correlates of Functional Activity

Objectives:

We are currently involved in perfecting high resolution, autoradiographic techniques for the cellular localization of metabolic activity in the normal animal and the elucidation of pharmacological influences on these neuronal systems. Correlation of these patterns of activity with the localization of pharmaco-receptor sites, and changes in these receptor systems due to pharmacological intervention, is underway using series of adjacent tissue sections.

Similar techniques will be useful in our metabolic studies of the development of functional cell systems which have been morphologically characterized. These methods enhance the resolution available to study the activity of neurons in the developing brain, particularly those hypothalamic systems involved in the development of rhythmicity in brain function.

Methods Employed:

We are developing techniques which will permit us to use [^{3}H]-2-deoxy-D-glucose (2-DG) as a metabolic marker of glucose utilization at the cellular level of resolution. Previous autoradiographic localization studies provided fairly gross regional indices of glucose uptake. The functional metabolic activity of individual

neurons requires better fixation of the diffusable 2-DG molecule to its uptake site. We hope to accomplish this by the fixation of 2-DG in situ using perfusion with a periodate-lysine-paraformaldehyde fixative followed by embedding in paraffin or soft plastic. The "PLP" fixative locks carbohydrates in place, and embedded sections prepared in this fashion prevent further diffusion of the labeled metabolite from the locus of utilization. These sections can be treated by routine autoradiography or exposed to tritium-sensitive LKB Ultrofilm for visualization.

Autoradiographic localization of opiate and phencyclidine receptors can be compared in the same animal with manipulation-induced alterations in brain metabolism measured by the 2-DG technique. Alternate sections from an animal previously injected with 2-DG are either processed for 2-DG autoradiography or for receptor localization. Removing the diffusable 2-DG by preincubation of sections in buffer permits receptor localization, while diffusion of the [^{3}H]2-DG label is prevented by quick drying and subsequent tritium-sensitive film exposure. In this way, the alteration of brain metabolism by anesthetics may be correlated with receptor binding in those brain regions affected.

Major Findings:

These techniques of 2-DG analysis have confirmed that phencyclidine-induced changes in brain metabolism predominate in limbic regions. A striking variation from normal neocortical glucose utilization is the relatively greater 2-DG uptake in the form of columns which appear in register with granule cell-poor zones of layer IV somatic sensory-motor cortex. The pattern of labeling suggests that uptake columns occur in cortical areas spared by inputs from thalamic relay for somatic sensation. Layer I of entorhinal cortex and the subiculum also shows a selective and dramatic increase in 2-DG uptake. Such increased metabolic activity may be correlated with the terminations of pathways from regions rich in phencyclidine receptors. These results suggest that phencyclidine influences brain metabolism by selectively affecting the activity of neo- and archicortical systems.

Significance to Biomedical Research and to the Program of the Institute, and Proposed Course:

The visualization by autoradiographic techniques of opiate receptor locations throughout the CNS has greatly advanced our appreciation of the richness of opiate functions in normal physiology and has opened a surprising number of doors to the investigation of receptor-mediated brain processes. We have just begun to appreciate how receptors influence and control neuronal development and the establishment of neural connections, of the interrelatedness of receptor subtypes and of neurochemically distinct systems (such as dopaminergic and opiatergic interactions in the striatum), of the evolution of receptors as markers of synaptic complexity and of the significance of species differences. We are encouraged by preliminary comparisons of drug receptors and the altered metabolic profile (as seen by 2-deoxyglucose autoradiography) produced by the same drugs. These findings indicate a productive future in our basic understanding of brain function. Of clinical importance are some preliminary data on receptor differences, visualized by autoradiography, in genetically "nervous" dogs. If these have a correlate in human mental disorders, as they appear to be pharmacological, then we will be encouraged to proceed with a program to analyze receptor distributions in the brains of deceased humans with well-documented histories of Parkinson's disease, Huntington's chorea or schizophrenia.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01091-04 LNP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Motor Function in Patients with Neuropsychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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1.50	1.50	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of this project are to examine the contributions of central motor programming and afferent input in the control of arm movements in normal and parkinsonian subjects, and to study psychomotor performance of patients with central motor disorders. The first set of experiments records electromyographic activity and kinematics of limb position while subjects manually match a target display with either a skilled rapid or slow movement with a handle whose displacement controls a visual display or attempt to control the discharge of single motor units (MU) when the limb is passively moved. Movement amplitude, presence or absence of visual feedback of position, and disturbances of the subject's movements are independent variables. Large movements and sustained rates of MU discharge are performed accurately independent of manipulation of the experimental variables but accurate performance of small movements and generation of single MU spikes becomes increasingly dependent on the absence of limb disturbances during movement. The second set of studies examined a variety of psychomotor variables from patients with Parkinson's disease. Tests of movement speed, reaction time, and gait abilities have provided measures that correlated with clinically determined fluctuations in drug efficacy.

I. Central and Peripheral Control of Movement in Humans.

Project Description:

The importance of afferent information in the control of limb movements is controversial. Whereas it is clear that afferents exert potent physiological effects on spinal motoneurons and cells in supraspinal structures, it has been suggested that some of these afferents contribute little to the final positioning of a limb. A case in point is the observation that muscle spindle activity does not reflect muscle length during rapid movements of large amplitude, thereby casting doubt on a regulatory role for spindles at the end of movements. In addition, physical disturbances imposed during movements, that likely activate muscle spindles, do not appear to modify final limb positioning. There are, however, other experiments demonstrating the importance of afferent input in a variety of tasks performed by humans. For example, ischemic deafferentation of limbs alters position sense and sense of effort. Furthermore, performance of fine motor tasks, such as reproduction of alphabetic characters is also disrupted by ischemic deafferentation and it is noteworthy that inactivation of the gamma loop in humans impaired the ability to tonically activate motor units, though phasic activation was not impaired. It is the object of the present project to continue examination of the role of peripheral inputs in the control of limb movements. Both normal volunteers and patients with neurological disorders will be studied during performance of movements of varying sizes. Different types of limb disturbances will be imposed during the movements. Two general experimental approaches are being pursued. In the first, the psychomotor variables of movement error and movement time are studied in relation to physical disturbances. In the second group of experiments, intra- and extramuscular electromyographic activity is examined when the limb is displaced while subjects perform motor tasks or maintain postures.

Methods:

Human subjects are trained to manipulate a handle that is attached to a servo-controlled torque motor while performing either supination-pronation forearm movements, extension-flexion of the wrist, elbow or index finger, or abduction-adduction of the index finger. Displacement of the handle causes movement of an oscilloscope beam that is to be matched by the subject with a second, experimenter controlled, oscilloscope beam.

In one series of experiments, subjects perform tracking movements either as rapidly as possible or as accurately as possible. For each of the three or four movement sizes (1°, 3°, 10°, and 30°) subjects are given approximately 250 training trials. Independent variables include (1) direction of movement, (2) presence or absence of visual feedback, (3) continuous loads opposing or assisting movement, (4) muscle fatigue, and (5) brief physical disturbances delivered to the arm before or after initiation of arm movement. Patterns of muscle activity and tracking errors are analyzed. For other work, single motor units are recorded from the dorsal interosseous muscle with microelectrodes. The first metacarpophalangeal joint is positioned over the axle of the torque motor, the index finger is wedged into a holder that prevents finger movement, and the thumb is immobilized in a fully extended position. All tests are done in the isometric situation. In one task, subjects seek to generate a single motor unit impulse, a task that requires a brief period of practice for each new motor unit. For a second task, subjects maintain a sustained rate (about 10 Hz) of motor unit discharge for 5 to 10 minutes. The torque motor is used to periodically stretch or shorten the DI during both of these tasks. A visual cue provides a signal calling on the subject to generate a

single impulse from a motor unit and on certain trials finger displacements are produced either at a fixed delay (50 to 150 msec) after the visual cue or immediately after the occurrence of the first motor unit impulse following the cue. During maintenance of a sustained rate of discharge, displacements occur either randomly or at a fixed interval in relation to the discharge of the motor unit. The effects of finger displacements on motor unit discharge are evaluated by comparing the proportion of successful attempts to generate single spikes and the number of impulses in the presence or absence of disturbances. Effects of disturbances on tonic motor unit discharge are evaluated by comparing the number of impulses before and after displacement of the finger.

Preliminary Findings:

Motor behavior of normal subjects and parkinsonian patients has been studied for this project. For the studies evaluating psychomotor performance the subjects have all been studied under no-load conditions when rapid movements were performed. Several findings have emerged:

(1) For both parkinsonian patients and normal humans the amount of relative error increases as movement size diminishes. Movement accuracy when velocity first reaches zero following movement initiation is usually less than a mean of 10-15% for the 30° movement, but can increase to 40-50% for a 3° movement. At attainment of final position, error is less that 5% for the 30° movement but often greater than 15% for the 3° movement. The presence or absence of visual feedback does not influence movement accuracy when velocity first reaches zero but somewhat increases error at final positioning when no feedback is available.

(2) In normal subjects limb perturbations at the onset of movement increases relative positional error. This effect increases as movement size diminishes, so that, for example, error is increased to about 20% versus 10% with no disurbances for 30° movements, and for 3° movements 60-80% versus 40-50% in the absence of perturbations. In addition, the limb's final position deviates from the target position when the limb is perturbed provided no visual feedback is available. Thus, for large movements the final positional error can be either less (i.e., positional undershoot) or greater (i.e., positional overshoot) than the spatial location of the target; for small movements the final position is invariably greater than the target position following disturbance of the limb.

(3) Muscle fatigue exacerbates tracking errors for movements generated by contraction of the fatigued muscle. In a fatigued muscle perturbations are even more effective in disrupting performance than in a normal muscle. In these experiments movements mediated by the unfatigued muscle are indistinguishable from those in the normal subject.

(4) When movements of patients with Parkinson's disease are briefly opposed, the movement tends to undershoot the target. Since patients with Parkinson's disease perform "rapid" movements relatively slowly, and often in a hypermetric fashion, the results obtained from this patient population must be carefully studied.

(5) Finger displacements modified both the proportionate success and the number of impulses when subjects attempted to generate single spikes from dorsal interosseous motor units. When the finger was not displaced subjects successfully generated one impulse in most of the motor units on more than one-half of the attempts. Successful control of five motor units was selectively disrupted by

passive DI stretch and reliable control of four motor units was selectively affected by passive shortening of DI. The successful generation of single spike in the remaining 10 motor units was disrupted by changes in DI length in both directions. For 22 of 26 motor units, the number of impulses was changed by displacing the finger. For 11 of these responsive motor units firing rate was changed almost exclusively during the displacing stimulus. For some motor units peripheral disturbances that shortened the DI evoked greater changes in discharge frequency than stretches of DI. The discharge frequency of another 11 motor units increased at short latency after ramp onset and the impulse rate then remained above zero for a variable period of time after DI was passively returned to the initial length. Two additional effects of delivering peripheral disturbances to the finger when subjects sought to generate one impulse were graded sensitivity to the amplitude/velocity characteristics of the disturbance, and the earlier occurrence (relative to the request) of impulses in 15 motor units when the DI was stretched.

(6) The effects of finger displacements on tonic maintenance of motor unit were weak. Nevertheless, the discharge frequency of 17/26 motor units was phasically modulated by displacement of the index finger during active maintenance of an 8-12 Hz frequency of DI motor unit discharge. For 12 of these 17 motor units the effects of DI motor unit discharge were confined to first 100 to 200 msec following onset of the stimulus. However, the modulation of motor unit discharge frequency of 5 motor units was often observed soon (less than 150 ms) after the return of DI muscle to its initial length. Sustained changes in discharge rate enduring throughout the displacing stimulus and beyond, were rarely seen.

(7) Motor units with a wide range of thresholds were studied. There appeared to be a tendency for low threshold motor units to be more sensitive to passive changes in the DI length at the velocities used in these experiments though there were several instances of low and high threshold motor units exhibiting nearly equivalent responsiveness to ramp stimuli.

II. Objective Psychomotor Evaluation of Neuropsychiatric Patients

Project Description:

In the last two annual reports we described the technical aspects and the use of a computerized system for obtaining objective, quantitative measures of motor function in patients with neurological disorders. Following the partial completion of the system in the summer of 1979, the measurement apparatus was installed in an appropriate setting for use in testing patients with neurological disorders in the Clinical Center. The system has been used to record and subsequently summarize data concerning motor functions in more than 50 patients with Parkinsonism participating in clinical trials of the experimental anti-parkinsonian drugs, lisuride, bromocriptine and pergolide. Patients with cerebellar disorders and essential tremor have also been studied. To establish normative data for movement variables measured on our system, thirty age-matched normal subjects have also been tested.

Major Findings:

Studies of age-matched normals revealed a considerable range of responses in the parameters measured by the system, with values varying between individuals as a result of such factors as physical stature, motivation, and temperament. Individual

scores, however, tended to be relatively consistent over time. Such expected outcomes as diminished speed of movement among members of the oldest age group when compared to younger normal subjects were observed.

Parkinsonian patients undergoing treatment with lisuride or bromocriptine were tested on a regular basis throughout the course of the buildup and placebo phases of the clinical trial. Grouped data, according to age, confirmed clinical observations in assessing motor performance in individuals undergoing treatment or evaluation. Immediate evaluation of patient performance is available to the test administrators at the completion of each test and periodic summaries of performance may be prepared easily. Furthermore, data collected in all studies to date has been permanently stored in computer files and is accessible for further study. These features of the system could be valuable for longitudinal studies of patients with neuropsychiatric disorders.

Significance to Biomedical Research and to the Program of the Institute:

Additional clarification of how tactile and kinesthetic sensory information is used to control skilled motor activity is essential to the understanding of normal and abnormal motor behavior in humans. It is hoped that these results will contribute to the understanding of how movements are initiated and completed. Finally, these studies will potentially develop standards of normal motor function and allow comparisons with patients with motor disorders to evaluate subclinical deficits and the efficacy of pharmacotherapeutic agents.

The objective evaluation of neuropsychiatric disorders that we have developed should prove useful in a wide variety of experimental applications that require computer recording and analyses of results. Long-term evaluation of patient's progress on medication regimens is particularly suited for objective analysis.

Proposed Course:

Future studies concerned with psychomotor performance will continue to investigate the importance of tactile and kinesthetic signals occurring during movements. Thus properties of movements and muscle activity will be investigated in normal and fatigued muscles, from functionally deafferented limbs, and following vibratory or mechanical disturbances delivered to a limb. A variety of movement types (e.g. large/small) and strategies (e.g. fast/slow) will be studied to determine the movements that depend upon sensory inputs from the periphery for accurate completion. More than two years of experimentation will be required to validate and extend the preliminary findings in providing additional information on the control of limb movements in normal and neurologically diseased humans.

The computer evaluation of movement is an ongoing project to provide objective evaluation of motor function in patients with movement disorders. Presently the neuroevaluation system is being used to evaluate the relative efficacy of pergolide and lisuride in treatment of Parkinson's disease. In addition to studying patients with parkinsonism, plans are underway to evaluate motor functions from a wide range of patients with motor disorders.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01092-03 LNP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Corticocortical Connections in the Sensorimotor Cortex of the Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	Michael Weinrich	Surgeon	LNP	NIMH

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.33	1.33	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project consists of a neurophysiological and neuroanatomical investigation of the organization of the somatic sensorimotor cortex and its role in the generation and control of primate motor behavior. One of the central questions concerning the organization of the cerebral cortex is how inputs to a cortical region from a variety of cortical and subcortical sources combine to contribute to its output. We have chosen to concentrate on the precentral motor cortex (MI), known to project directly to spinal motor neurons, and those cortical areas in close functional association with MI, the supplementary motor cortex (MII); area 3a (a transitional region between MI and the postcentral somatic sensory cortex), and the premotor cortex (area 6). Two related approaches have been adopted for this project: (1) an analysis of the organization of peripheral inputs to the premotor cortex, MI, MII, and area 3a and (2) single unit studies of cortical areas which supply inputs to MI.

Objectives:

The inputs to the precentral motor cortex (MI) as well as its intrinsic neuronal circuitry, determine the output of MI projection neurons, including those projecting to the spinal cord. Since there are a finite number of sources of afferent input to MI, as determined by neuroanatomical methods, it is a feasible objective to investigate neuronal activity within these sources during motor behavior, especially those behaviors for which MI activity is best understood. As a result of these studies we hope to gain an understanding of afferent interaction in producing motor cortex output. Ultimately, an objective of this project is to examine the activity of neurons which project to and from MI and to identify these neurons by antidromic axonal stimulation.

Two more general objectives of this project are (1) an improved understanding of the evolution, organization, and role in voluntary behavior of the entire motor cortex, a region which is likely to include, in addition to its "core," the MI cortex, a surrounding neocortical "belt" containing two or more representations of the motor periphery, including MII and the premotor cortex, and (2) a better understanding of the specialized role in sensory guidance of movements control of the somatic sensorimotor cortex.

Methods:

Training. Monkeys (Macaca mulatta or M. fascicularis) were conditioned to perform, with rigid head fixation, one of four tasks, depending on the experiments. (1) Monkeys were trained to make dorsiflexion (upward) or plantarflexion (downward) movement of one foot upon receiving a visual cue. A motor coupled to a foot pedal delivered abrupt changes in torque. For each unit, its relationship with movements and peripheral stimuli was evalated using the usual correlational and statistical criteria. (2) Monkeys were adapted to having a foot attached to a pedal. A servo-controlled motor established the position of the pedal at any of five positions and passively moved between these positions at one of four angular velocities. The relationship of each unit to the velocity and amplitude of passive foot displacements was evaluated using peak and sustained neuronal firing frequency. (3) A rhesus monkey was operantly conditioned to depress one of four keys located in a perimeter at arms length. While the monkey pressed one key, another of the four keys, selected randomly, was illuminated after a randomly varied delay. This key thereby became the next target. A barely discernable visual cue near the target key, appearing after another variable delay, signaled the monkey to move and depress the target. The monkey was required to make the movement within a short period of time, near the limit of reaction time. (4) The results of experiment #4, led to the design of a related experiment, currently in progress, which attempts to better characterize neurons within the premotor and motor cortex with respect to their relationship to the position of the limb, its velocity and acceleration, their role in visually guided and visually targeted movements and their responses to instructions concerning upcoming movements. The monkey has been conditioned to align two spots of light on a tangent screen in front of the animal. One of these spots is controlled by the computer (target), the other by arm movements of the animal. He must align the spots within a small accuracy "window" until the target light jumps to one of six locations. The monkey must maintain his arm position until the target light dims, at which point he must move (flex or extend his forearm) rapidly and accurately to the target position to align the light again and receive a reward. The sequence is then repeated.

For the first three experiments, extracellular unit activity of neurons in the somatic sensorimotor fields (area 3a, MI, MII, the first somatic sensory cortex (SI), and the premotor cortex) was monitored, recorded on magnetic tape with an 8-channel tape recorder, and analyzed off-line with PDP-12 and PDP-11 computers. For the premotor cortex experiments (see #'s 3 and 4 above), eye position was monitored using an electrooculogram (EOG). For experiment #4, the single unit activity was collected on-line with a PDP 11/03 computer using programs developed in this laboratory by William Stuart and modified by us for on-line control of the experimental paradigm, as well as the collection of single unit and behavioral data. Much of the past year has been devoted to establishing these on-line systems. They will allow greater flexibility in experimental design modification as well as simultaneous on-line acquisition of 2-4 channels of analog data in addition to the single unit activity.

Following the recording procedures, small amounts (5-10 μCi) of [3H]-amino acid have been and will be injected into either the premotor cortex, MI or MII. By noting the ultimate distribution of radioactivity in the brain, the sites of termination of neurons in the somatic sensorimotor cortex can be determined by tissue autoradiography.

Experimental Findings:

About 1600 units have been studied in five monkeys examined in this project to date; the examination of the input organization of the rostral sensorimotor cortex is nearing the completion of its initial phase and three reports on this work have recently been published. Several findings and interpretations have been developed:

1. MII is virtually unresponsive to peripheral inputs, as compared with MI in the same monkey. This finding is somewhat surprising from a neuroanatomical perspective, since MII receives monosynaptic corticocortical input from most divisions of the somatic sensorimotor cortex. However, the lack of profound somatic sensory responsiveness of MII neurons supports the hypothesis that MII plays a role in centrally generated motor programs rather than movements regulated by peripheral feedback.

2. MI, which is very sensitive to peripheral inputs, can be divided into at least two regions, one rostral and the other caudal, on the basis of the broad submodality type of its peripheral inputs (e.g., skin receptors vs. muscle receptors). These results suggest that cutaneous inputs to MI, since they predominate in a discrete (caudal) part of MI, may be more important in cortical motor control than usually believed.

3. Both area 3a and MI neurons receive information concerning both the velocity and position of parts of a limb.

4. These findings have enabled us to improve the current understanding of cerebral localization in this part of the cortex and the relationship of physiologically defined cortical regions to those defined by anatomical methods. Two of these points are most noteworthy: (a) microelectrode methods reveal that the boundary between MI and MII corresponds to the boundary between two anatomically defined parts of the agranular neocortex (termed areas 4 and 6) and (b) area 3a, the transitional field between the agranular and highly granular somatic sensorimotor cortex, appears to be, as it was originally defined (in the work of C. Vogt and

O. Vogt, 1919), a discrete cortical field characterized by a thin granular layer which receives inputs from muscle receptors concerning the velocity and, to a lesser extent, position of a limb.

5. The study of premotor cortex is in its preliminary phases, but some initial observations can be summarized. Most isolated neurons in the premotor cortex show changes in activity markedly before the onset of a voluntary movement and usually continue their altered discharge rate throughout the movement and for only a short period of time thereafter. Their activity is often specific for the direction of arm movement. These neurons are located within the frontal agranular cortex, corresponding to a part of area 6 as defined by the absence of a large population of giant, layer V pyramidal cells in addition to the lack of an internal granular layer (layer IV). The premotor cortex can also be distinguished from the MI representation by its markedly increased threshold for intracortical microstimulation effects. And finally, a substantial population of neurons change their activity in relation to motor set and/or signals which indicate the location of motor targets, and many of these are also specific for the direction of upcoming movements.

Significance to Biomedical Research and to the Program of the Institute:

Though it is often assumed in the neurological and neurobiological literature that different cortical fields within the somatic sensorimotor cortex relay specific information (e.g. sensory signals) between each other via corticocortical connections and that these cortical fields subserve different functions, there is very little direct evidence which supports this conclusion. Because of the potential significance of cortical specialization and localization on the mechanisms of normal and pathological motor behavior of primates and other mammals, the interrelationships between defined cortical fields deserves a detailed examination.

Proposed Course: In order to further examine the function of cortical connections, we must acquire more knowledge about the input-output organization of the cortex and the differential roles of the various fields within the somatic sensorimotor cortex. There is also a need for more reliable methods with which to distinguish the cortical fields from each other, especially in awake behaving animals. This project will be continued and developed in the Laboratory of Neurophysiology with the collaboration of Drs. M. Weinrich, and K.-H. Mauritz.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01093-03 LNP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Role of Somatic Sensory Inputs in the Cerebral Control of Movements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.83	1.83	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is a study of the role of sensory inputs in the cerebral control of motor behavior in the primate. The work described here is a comparison of neural activity in the somatic sensory cortex (SI) and the precentral motor cortex and a test of the feasibility of using two independently moveable microelectrodes to simultaneously record the electrical activity of single neurons in these two separate cortical regions. This technique should be useful in determining the characteristics of neurons in SI and MI that send and receive corticocortical projections. Such information might provide some clues as to the pathway taken by peripheral inputs to MI and its role in the initiation and control of movement.

Project Description:

Objectives:

Though peripheral inputs have been shown to affect MI neurons during movement, the significance and source of these inputs remains unclear. The previous experiments in this project addressed these problems by examining neuronal activity in those SI areas which are known to be densely and reciprocally connected to MI. It was found that the activity of many neurons in posterior SI regions showed a striking similarity to MI neuronal activity in terms of its relation to limb position and exerted force. While such a similarity is consistent with the hypothesis that peripheral inputs to MI are relayed through SI, it is equally consistent with the possibility that some SI neurons receive inputs from MI and are involved in the initiation of movement. One method to help distinguish between these two possibilities would be to simultaneously place two electrodes into the cortex, one each in regions of MI and SI with similarly behaving neurons. Then, a direct comparison can be made between neurons in these areas. Furthermore, by stimulating through one electrode and recording with the other, it should be possible to recognize neurons with corticocortical projections by the antidromic activation of their axons. The purpose of this project was to compare MI and SI neurons and to study the feasibility of the dual electrode technique.

Methods:

Two macaque monkeys were seated in a chair with their arms held by a plexiglass sleeve to a servo-controlled torque motor. One monkey was trained to flex or to extend its arm (around the elbow) while the other was trained to pronate or supinate its wrist. Alternating active movements of 20° were made with or against a steady torque which was applied by the torque motor and were followed by periods of steady maintenance of limb position. Sensory stimuli consisting of ramp displacements of the limb (10°) were delivered during the period between movements.

Following training, a chamber was attached to the monkey's skull and bolts were implanted for head immobilization. Single unit activity was recorded from neurons in the forelimb region of the somatic sensorimotor cortex. To aid in the initial definition of MI, SI and adjacent cortical fields, a simple and efficient method for the delineation of the general somatotopical organization of the cortex within a chronic recording chamber was developed. Briefly, this technique utilizes stainless steel ball electrodes to apply monopolar anodal pulse stimulation to the exposed dura of an anesthetized monkey. By stimulating at different cortical sites and noting the movement evoked and its threshold, the MI representation of the arm, trunk and leg could be determined.

The technique for recording single units with two independently moveable electrodes utilized two Trent Wells hydraulic microdrives mounted on separate plastic plates. This system was designed and fabricated by Mr. James Sullivan, Biomedical Engineering and Instrumentation Branch, after discussions with Drs. Evarts, Jennings, Donoghue and Wise. Several plates were available each with a different distance between the hole through which the electrode passed and the edge of the plate. Thus, any desired distance between electrode penetration sites could be achieved by choosing the appropriate plates and clamping them in the desired configuration on the chamber.

Corticocortical connections were searched for by advancing a recording electrode

(usually in MI) while electrically stimulating through the other electrode (usually in the caudal part of SI). Single negative pulses of 0.2 msec duration and 10 - 60 uamp current intensity were used. These pulses were immediately followed by 0.1 msec positive pulses in order to reduce stimulus artifacts. A search was made for two classes of neurons, those antidromically and orthodromically activated. Antidromically activated neurons were characterized by their short fixed latency of activation and collisions with spontaneous or evoked spikes when present. Orthodromically activated units were characterized by longer and, more importantly, variable latencies of activation.

Unit activity and event marker codes were recorded on-line with the equivalent of a PDP-11/03 computer. In addition, handle position, velocity, EMG, codes and unit activity were recorded on magnetic tape for later analysis. For each unit analysis focused on the frequency and pattern of discharge at each steady-state position and force and the change in these parameters that occurred during passive displacements (sensory stimuli) and active movements.

Results:

Comparison of SI and MI Neuronal Activity

Analysis of single unit activity recorded for 1612 neurons in two monkeys have, thus far, produced the following important similarities and differences between SI and MI.

1. Both SI and MI neurons were organized into zones with predominantly cutaneous or non-cutaneous inputs. The cutaneous zone in MI (MI/c) was located in the bank of the central sulcus, while the cutaneous zone in SI included areas 3b and 1. The non-cutaneous zone in MI was rostral to MI/c (this zone was termed MI/r) and in SI (the non-cutaneous zone) consisted of areas 3a, 2 and 5.

2. The cutaneous neurons in MI and SI were similar to each other in their lack of sensitivity to maintained force or position and to the direction of active and passive movements. In addition, MI and SI neurons with non-cutaneous inputs were similar to each other, but different from cutaneous units in their sensitivity to maintained force and position and to the direction of active and passive movements.

3. In contrast to their similarity during maintained force and position, many of the non-cutaneous SI and MI neurons showed very different patterns of activity during transitions from one state to another. The activity of most MI neurons resembled the pattern of EMG activity during changes in force and position. For example, a unit with increased activity related to maintained supination would usually be characterized by a phasic increase in activity during a supination movement. In contrast, some SI neurons showed the opposite pattern, e.g., phasic decreases in activity during movements towards positions associated with tonic activity increases.

Dual Electrode Recording System

Neurons with corticocortical connections were searched for in 56 pairs of simultaneous electrode penetrations in SI and MI. Most MI penetrations were located in regions where the activity of the units investigated was related to the task as

judged by the usual correlative criteria. Particularly well related neurons, e.g. units whose activity increased for one direction of active movement and decreased for the other were also encountered in these penetrations. Most penetrations with the stimulating electrode were made in SI regions where the neurons had cutaneous receptive fields on the hand or forearm or, in some cases, non-cutaneous receptive fields associated with the wrist or elbow. Several stimulation effects were noted:

1) Following stimulation through the electrode in SI, there commonly was a dramatic and long lasting (up to 100 msec) inhibition of ongoing single unit activity in MI. In most cases the background activity also decreased, suggesting that the corticocortical inhibition was a general phenomenon affecting many MI neurons. Consistent with this was the observation that these inhibitory effects could be produced from widespread stimulation sites.

2) MI units were orthodromically activated. About 20 of these units were observed with latencies between 1.5 and 8 msec. Such effects could only be obtained from stimulation sites in a much smaller cortical area than those producing the above noted inhibitory effect. Insufficient information was collected concerning these units to determine whether they differed overtly from non-orthodromically activated units during the task, though further analysis is in progress.

3) A few units were antidromically activated. Only 3 of these units were recorded despite exhaustive attempts to stimulate and record at a variety of penetration sites and depths within each penetration. In addition, stimulation through low impedance electrodes (about 0.5 MOhm) was attempted (to increase the amount of current spread) without effect.

Several explanations for the rarity of observed corticocortical neurons, despite their known existence in high concentration in these cortical areas, can be postulated. The cells may have been too small to isolate with our electrodes. They may project to a very restricted target zone. The antidromic potentials may have collided with faster, stimulation evoked orthodromic potentials. Or perhaps we employed stimulation parameters inappropriate for our electrodes (although the same stimulation methods have been used successfully with similar electrodes in acute preparations).

In conclusion, several aspects of the double electrode technique need further development for application to chronic neurophysiological preparations. In particular, it might be useful to develop techniques for implantation of arrays of stimulating and recording electrodes in order to determine the distribution of antidromically activated units in MI, while stimulating in SI.

Significance to Biomedical Research and the Program of the Institute:

Further clarification of how sensory information from the periphery is utilized to initiate and control skilled motor activity is essential to a better understanding of normal and abnormal movements in man. Though much is known about the involvement of motor cortex in motor control, more information is needed concerning the characteristics of sensory input to MI and, in particular, the role of corticocortical inputs from SI. As an important step towards this goal, the present project tested the feasibility of using two independently moveable electrodes to examine the characteristics of neurons sending or receiving corticocortical projections.

Proposed Course of Project:

Analysis of the differences between cortical areas in the response of their neurons to sensory stimuli and their activity during active movements will continue. However, more needs to be learned about the technical aspects of a two electrode recording system before such a technique will be useful in an awake, behaving monkey. The potential benefits in terms of understanding interactions between different cortical regions may warrant this continued effort, but in the immediate future further analytical study of sensory inputs to SI and MI and cortex will be the focus of this project.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01094-01 LNP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Information Processing in the Motor Cortex.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.83	.83	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this three-part project is to examine the functional organization of neurons in the motor cortex. Rats have been chosen as the primary experimental animals since they are readily available, easy to condition, and have small lissencephalic brains. (1) Neuroanatomical and neurophysiological techniques will be employed to characterize the first motor cortex (MI) in rats and determine the connectional relationships of MI with other neural structures. (2) We will record from several classes of projection neurons in the forelimb area of MI cortex in awake, behaving rats. In these experiments, the relationship of the neuronal activity to motor output will be examined for each identified class of neurons, e.g. corticospinal cells and corticostriatal cells. (3) Intracellular recording will be combined with intracellular injection of a tracer, horseradish peroxidase, to visualize the distribution of neuronal processes, in different layers of MI cortex, of identified projection neurons. These experiments are directed toward an understanding of information processing within the motor cortex, in particular the mechanisms of the input-output functions of the motor cortex.

Objectives:

The output of neurons in the somatic sensory-motor (SSM) cortex is closely linked to motor activity in mammals. These cortical outputs arise after intracortical processing of peripheral and central inputs through a complex array of neurons. The experiments described here are designed to elucidate the role of different cell types in the input-output transformations that occur in first motor (MI) subdivision of SSM cortex.

The first requisite step in these studies is to better define the first motor (MI) subdivision of the SSM cortex. This can be accomplished by employing neurophysiological mapping techniques in conjunction with neuroanatomical pathway tracing and cytoarchitectonic techniques. The second objective is to examine the role in motor cortical function for each of several different classes of projection neurons in MI cortex during a simple forelimb motor task. Previous studies have shown that cells in the superficial layers of MI cortex project mainly within the cortex while cells in the deeper layers project mainly to diencephalic, brainstem, and spinal targets. However, except for pyramidal tract neurons, there is little known about the contribution of different types of cortical projection neurons to cortical output or to the control of motor activity. The third objective is to identify the intracortical connections of these identified cell types. Thus, one can evaluate potential routes of information flow within small modules of neocortex and better understand the mechanisms that produce cortical output signals.

Methods:

1. Identification of MI cortex. The MI cortex is localized by mapping the cortex with intracortical microstimulation (30 msec trains, 330 Hz, 50 usec pulses) and multi-unit recording techniques. MI can be identified by the ability to produce movements by low threshold stimulation (<65 ua). Lesions are placed at sites of transition from low to high threshold or a change in peripheral response properties. The animals are then perfused with formaldehyde and the cytoarchitectural characteristics of cortical regions are determined. For pathway tracing experiments, axonal tracers (histochemical markers or radioactive amino acids) are injected into neurophysiologically characterized cortical regions. Following appropriate survival times the animals are perfused and the brain processed by standard methods to reveal the distribution of tracer substances in the brain and thereby the connections of the injected cortical regions.

2. Chronic recording. Water-deprived rats are trained to press a bar or maintain a constant force with their forelimb in order to obtain their water reward. On the bar pressing task, the rat must close a switch by moving a lever several millimeters. In the constant force (isometric) task the animal must maintain a force level with its forelimb against a stationary lever for a given period of time, then decrease its force output to receive a reward. After learning one of these tasks, a recording chamber is placed over the forelimb region of MI cortex and single unit recordings are made during task performance.

Unit activity, force, and bar pressing are recorded on-line with a PDP 11-03 computer and these data are analyzed off-line. Following these initial experiments, stimulating electrodes may be placed in the corticospinal tract, basilar pontine nuclei, red nucleus, thalamus and the contralateral cortex and striatum to identify, in order to test for antidromic activation of the MI neurons that project to these structures. The location of recording and stimulation sites are verified in histological sections from these animals.

3. Intracortical circuitry The intracortical connections of neurons in MI cortex are identified by intracellular recording and subsequent labeling of single neurons with horseradish peroxidase (HRP) in anesthetized animals. Intracellular recordings are made with glass electrodes filled with 4% HRP in tris/KCl buffer. The distant axonal connections of each neuron are determined with antidromic activation methods. Stimulating electrodes are placed in the pyramidal tract, corticospinal tract, contralateral cortex, and in some cases, in other subcortical targets of MI neurons. Each antidromically identified cell is injected with HRP. At the termination of the experiment, the animal is perfused, the tissue is sectioned with a vibratome, and the distribution of tracer within the cell is demonstrated with standard histochemical techniques. The morphology of the injected cell and its processes is reconstructed with a microscope and drawing tube (camera lucida).

Significance to Biomedical research and to the program of the Institute. Elucidation of the mechanisms of cortical information processing in the motor cortex, especially the role of different cell types in affecting cortical output, will provide a better basis for understanding normal and abnormal motor and sensory function in man.

Progress Report and Preliminary Experimental Findings.

1. Mapping and Pathway Tracing experiments. Retrograde axonal transport experiments demonstrate that the corticospinal projection originates, in part, from two cytoarchitecturally distinct fields of the frontal agranular cortex (FAC). Anterograde transport experiments show that fibers from FAC terminate in the ventral horn of the spinal cord, in addition to a dense termination within the intermediate spinal laminae. These findings suggest that both of these FAC fields are part of the SSM. Under ketamine anesthesia, low threshold microstimulation elicits movements of the contralateral head, trunk, and forelimb from only one of these FAC fields. The low threshold field can be characterized by the relative homogeneity of its superficial laminae; which contrasts, in thionin stained sections, with the less densely staining layer III of the adjacent higher threshold FAC field. We therefore conclude that the low threshold FAC field corresponds to the MI cortex, while the higher threshold, spinally projecting part of the FAC, is a distinct cortical field within the SSM. These experiments demonstrate that a cytoarchitecturally distinct cortical field can be identified as a major part of the MI cortex in the rat.

2. Chronic recording experiments. For chronic recording experiments six rats have been trained to bar press. A recording chamber and microdrive system suitable for rats has been designed, built and tested in anesthetized rats.

3. Intracellular experiments. We have designed a new intracellular recording microdrive/stereotaxic frame assembly and an intracellular amplifier that are currently being fabricated. Other necessary equipment has been acquired and assembled.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01335-11 SMRA

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
22	18	4

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The following report summaries the extensive studies of schizophrenia performed by the Adult Psychiatry Branch during the reporting year 1980-1981. The report is divided into the major headings of:

I. Psychology
II. Psychophysiology
III. Biochemistry and Pharmacology
IV. Indoleamines
V. Catecholamines
VI. Neuroanatomy

All studies are briefly summarized under subheadings within the appropriate section.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To investigate the process of schizophrenia examining its effects and exploring its etiologies and its essense. Our work in this area attempts to produce new knowledge while synthesizing prior information in the psychological, physiological, and biochemical disciplines toward understanding and treating schizophrenia.

Methods:

To achieve our objectives, a wide variety of studies are undertaken in the laboratory. The methods for each study are detailed in the appropriate section. All subjects in our studies of schizophrenics are phenothiazine-refractory chronic schizophrenics. Controls are appropriately matched. When drugs are administered and measurements determined, the method is double-blind. The utmost care is given to ensuring that the studies are objective and reliable. Only then will the results yield a fuller understanding of schizophrenia and improved methods of treatment of this perplexing disorder.

I. Psychology

The Rating Scales: Rating scales are an essential tool in clinical investigations. Refining those scales so that they are objective, relatively brief, comprehensible, and accurate representations of psychopathology is an ongoing process. That process involves frequent evaluations of the scale with the nurses and physicians who use them and the statisticans who help to interpret them. We present a brief overview of our latest results with these scales.

Inpatient Behavioral Rating Scale: The Inpatient Behavioral Rating Scale (IBRS) is an inventory designed to record daily nursing observations of mood and behavior of patients in a clinical setting. It consists of 26 items characterizing a broad range of psychopathology and is particularly suitable for use where change in mood and behavior is relevant to clinical investigation. It is formated on an optical scan sheet for ease of computer entry and analysis.

Brief Psychiatric Rating Scale (BPRS): Brief Psychiatric Rating Scale originally introduced by Overall and Gorham in 1962, has been extended and descriptive anchor points provided through a joint effort by Drs. Llewellyn Bigelow and Dennis Murphy. The scale has been shown capable of accurately reflecting the cycles in a patient with a periodic psychotic illness. Additionally, the scale instructions have been shown to be remarkably reliable. This was established by having three psychiatrists of diverse backgrounds, naive to the scale, rate 15 patients independently in an interview setting. The intraclass correlation coefficients obtained from this exercise were impressive. We are in the process of writing up this scale for publication. Since the original Overall and Gorham BPRS has found extremely wide application, we anticipate that our scaled version will gain considerable popularity and use.

Premorbid Adjustment Scale: This scale devised by Eleanor Cannon-Spoor and Steven Potkin, was rendered objective and reliable by our standardized procedures. It

was found to successfully differentiate a variety of groups. Normals, schizophrenics and those with a schizoaffective disorder were separated from each other using this scale. Among the schizophrenics, differentiation on the basis of subdiagnosis was achieved only on the "late adolescence" and "adulthood" subscales for chronic paranoid versus chronic undifferentiated schizophrenics. Total premorbid adjustment score was significantly correlated with length of hospitalization. Further, the scale was found to differentiate between patients who had been chronically hospitalized for at least seven years versus patients who were on an outpatient basis at the time of rating. The scale also differentiated patients who had had an insidious onset of schizophrenia from a group whose onset had been acute. In a study of CAT scans done on chronic schizophrenics, the Premorbid Adjustment Scale distinguished between patients with normal versus abnormal CAT scans.

In addition, a study of chronic schizophrenics in India has recently been completed in which the premorbid scale was used. The scale was modified slightly for this purpose in order to more accurately reflect cultural differences. We are currently examining these results.

Wechsler Adult Intelligence Scale (WAIS): The WAIS is a relatively standaralized tool to measure ways of thinking. We recently used it to explore the effects of certain drugs. Parkinsonian patients treated with L-DOPA had decreased scores over time on the Comprehension substest of the Wechsler Adult Intelligence Scale (WAIS), whereas scores on all other ten subtests of the WAIS increased. It was hypothesized that if L-DOPA treatment, which increases dopamine activity in the brain, is directly related to an apparent deleterious effect on the WAIS Comprehension, then a drug such as alpha-methyl-para-tyrosine (AMPT), which decreases dopamine activity, might have an augmenting effect on this subtest. A therapeutic trial of AMPT in a group of chronic schizophrenic patients provided an opportunity to test this hypothesis. It was found that Comprehension scores improved significantly with AMPT. Other clinical rating instruments failed to show any changes.

Scores from 94 psychiatric and neurological patients on the 11 subtests of the WAIS and the Halstead-Reitan Category and Finger Tapping tests were used in a factor analysis. The Category Test had a low loading on the first verbal factor and a high loading on the second, visuomotor factor; the Tapping Test had a high loading on a small fourth factor of manipulative speed. The finding with the Category Test suggests that it, contrary to the expectation of many of its users, does not distinguish an ability that is both separate from nonverbal intelligence and particularly sensitive to all types of brain pathology. It is concluded that visuomotor and verbal factor scores might be more efficient than conventional scores in studies of effects of brain damage.

Halstead-Reitan Neuropsychological Test Battery: The Halstead-Reitan Neuropsychological Test Battery is an extensive collection of neuropsychological tests, an essential tool for us to use in examining the intricate blend of psychology and neurology in our investigations of the mental process.

Four of 17 chronic schizophrenic and schizo-affective patients between the ages of 20 and 35, in partial remission and living in the community, were found to have mildly or moderately prominent sucli, as disclosed by computerized tomography (CT

scan). These four patients were matched for gender and age with four chronic schizophrenic or schizoaffective patients without sulcal prominence. The Halstead-Retian battery (HRB) of neuropsychological tests, including the Wechsler Adult Intelligence Scale (WAIS), was administered to these eight patients with the examiner blind of results to the CT scan. Each of the four patients with sulcal prominence considerably exceeded an established cutoff score for brain impairment on the HRB, whereas only one of the control schizophrenics performed in the impaired range, just beyond the cutoff. The WAIS Verbal IQ, measured concurrently, was in the normal range for all subjects and similar for the two groups. These findings suggest that mild or moderate sulcal prominence on CT scans among young chronic schizophrenics is associated with impaired neuropsychological functioning.

A second study, including the HRB and WAIS, attempted to identify blindly those patients with evidence of morphological brain abnormalities on prior computed tomography (CT). The CT scan status of 12 of 15 (80%) patients was correctly identified solely on the basis of neuropsychological testing. These results supported our hypothesis that impairment on the HRB in chronic schizophrenic patients was associated with morphological abnormalities on the CT scan, and that the positive and negative CT scans of these patients could be predicted accurately.

Bender-Gestalt Test: The Bender-Grestalt test is a measure frequently employed to examine the organic nature of mental processes. The B-G was administered to 20 schizophrenic patients, ten with tardive dyskinesia (TD) and ten without tardive dyskinesia. Blind assessment of the patients' reproductions on two different occasions revealed a high test-retest reliability for the ratings. Significantly more TD patients (9/10) had organic signs than the non-TD patients (4/10).

II. Psychophysiology

A. Sleep: Our laboratory has been involved in the exploration of sleep for quite some time. The sleep of 13 drug free chronic male schizophrenics was studied during a one-month trial on chlorpromazine (CPZ) and compared with one-month placebo periods. Recordings were made only after subjects had been on the drug or placebo atleast three weeks. Sleep latency and awake times were significantly less on CPZ while Stage II, Delta Sleep, Delta %, Non-REM activity, and REM Density were significantly increased. There were no significant changes in REM Latency, REM time, REM %, Stage II %, and Non-REM %.

We investigated whether schizophrenics have a normal REM rebound following REM deprivation. In addition, since Cartwright et al. previously suggested that "good REM compensators" tend to be field independent, we related a measure of field independence (the portable rod and frame test) to degree of compensation.

Eight actively ill schizophrenic patients (E) (5F, 3M mean age = 25) and eight non-psychotic control patients (C) (5F, 3M mean age = 26) were partially REM deprived by the awakening method for two nights. Sleep on each of five Recovery nights (R_1-5) was compared with that of Baseline Sleep. All patients were unmedicated except for a single control patient who received chlorpromazine.

On R_1 mean change in REM compared with Baseline was +38 min for C and -2 min for E ($p < .01$); on R_2 it was +17 for C and -10 for E ($p < .05$). Mean change in REM during all Five Recovery nights was +14.5 min for C and +1 for E ($p < .05$).

Mean Baseline Sleep patterns (min) were similar in E and C patient group

	Total Sleep	REM Sleep	REM Latency	REM %	Stage III - IV
E	378	93	72	25.0	37
C	390	99	78	25.5	32

Mean loss of REM was 71.8% for E and 72.8% for C. Controls had to be awakened significantly more frequently than E (35 awakenings compared with 28, $p < .05$).

A two-way multivariate analysis of variance--one factor being patient groups, the other being a repeated measure, linear trend analysis--revealed that C had signifi cantly different sleep patterns than did E during Recovery ($p < .03$, $F=2.45$). The major source of difference was in REM sleep recovery ($p < .006$, $F=8.06$) but, surprisingly, Stage III + IV was also a significant contributor ($p < .02$, $F=6.13$). E tended to have elevated Stage III + IV on the first two Recovery nights and low and normal amounts on the last two except on the last Recovery night when Stage III + IV tended to be elevated, though not significantly.

REM rebounds during Recovery were correlated with increased Total Sleep ($r=.52$, $p < .01$). Nevertheless, after removing the effects of change in Total Sleep during Recovery by analysis of co-variance, the controls still had the normal pattern of REM rebound and the actively ill schizophrenic patients did not, i.e., the two groups differed in rate of REM decline over the five Recovery nights ($p < .03$).

Among four E and four C patients tested, there was a significant correlation between degree of REM rebound and field independence ($p < .05$, $rho=.79$). Mean change in REM during Recovery was 27 min for three field independent patients and -1.5 min among five field dependent subjects ($p=.036$, Mann-Whitney U-test).

These results suggest that actively ill schizophrenics differ from other psychiatric patients in failing to have a normal REM rebound. This difference was not attributable to differences in age, sex, baseline sleep, anxiety, or change in Total Sleep time during Recovery. REM compensation may also be correlated with field independence, although further research needs to be done to substantiate this finding before any conclusions may be drawn.

B. Evoked Potentials and EEG: We studied the evoked potentials of 21 schizophrenics and 21 controls. They were matched for age and exposed to a 10-minute sequence of frequent and infrequent auditory stimuli. The amplitude of P_3, a response component of the evoked responses to infrequent stimuli, was much larger in the control group ($p < .0003$). Using a P_3 amplitude of 3.20 V in the first 2.5 minutes as a dividing point, only seven of the 42 subjects were misclassified. This amplitude had a significant correlation of 0.48 with the patient's age at first hospitalization. The group P_2 amplitude to frequent stimuli was also much larger in the control group

($p < .003$). This amplitude has a significant correlation of -.41 with medication dosage in terms of equivalent amounts of chlorpromazine. Behavioral scales failed to correlate significantly with evoked response measures. Evoked response latencies did not distinguish the two groups.

A study is underway to develop a means of demonstrating whether or not schizophrenic patients have an unusual lateralization of brain functioning. Visual processing of verbal and non-veral tasks is being tested to show if patients have recognizable differences from normal subjects. The tasks involve the use of a projection tachiostoscope and are being presented to normal subjects to establish normal patterns of response. Patients are being tested also, but inconsistent results have developed from limited trails. Should the theory behind this study prove workable, it could be applied to tests of various drug effects, used to monitor the progress of treatment of patients, provide information about a possible specificity in mental dysfunction.

III. Biochemistry and Pharmacology

IV. Indoleamines

A. Indoleamines and Related Compounds: The indoleamines and related compounds have been hypothesized to play a role in the process of schizophrenia. Our laboratory has explored the role of serotonin, DMT, and other related compounds using a variety to methods of elucidate the nature of the relationship.

1. Serotonin: L-5-hydroxytryptophan (L-5HTP) and the peripheral decarboxylase inhibitor carbidopa were administered to chronic schizophrenic patients in three separate experiments using a double-blind, placebo-controlled cross-over design. The three experiments were: (1) L-5HTP administration to 15 patients who had been withdrawn from all neuroleptic medication; (2) L-5HTP administration to seven patients maintained on haloperidol; (3) L-5HTP administration to nine patients maintained on chlorpromazine. Although the groups were diagnostically homogeneous, individual responses were highly variable. Considering each group as a whole, the only significant changes in rated psychosis consisted of an increase in the first group consequent with the effects of neuroleptic medication withdrawal and an increase in psychosis scores associated with adding L-5HTP to chlorpromazine. Neuroleptics apparently sensitize the central nervous system to the effects of L-5HTP loading. Acute exacerbations of psychosis induced by L-5HTP can be reversed by neuroleptics. Our experience does not give encouragement to the hypothesis that schizophrenic illnesses arise consequent to a functional deficit of serotonergic function that can be treated by giving a serotonin precursor in pharmacological quantities.

Whole blood serotonin concentrations were studied in 33 chronic schizophrenic patients previously having had CT brain scans and 23 healthy volunteers. The chronic schizophrenic patients had a mean serotonin significantly higher than the controls.

The patients were subdivided into those with abnormal CT scan findings (enlargement of cerebral ventricles and/or cerebral atrophy) and a group with normal CT scans. The patients with abnormal CT scans had significantly higher serotonin concentrations when compared to schizophrenics with normal CT scans as well as

controls. The chronic schizophrenic patients with normal CT scans did not have significantly elevated serotonin concentrations compared to controls. Furthermore, ventricular size in the total patient group was significantly correlated to serotonin concentration.

2. N,N-Dimethyltryptamine (DMT)

a. Behavioral

N,N-Dimethyltryptamine (DMT) is an endogenous compound, found in human tissue. It may be differentially elevated in psychiatric patients. When given to man, DMT produces psychological disturbance. Instead of trying to find an animal equivalent of schizophrenia, we studied the behavioral effects of this compound in rats and applied to these behaviors criteria derived from the clinical condition of schizophrenia. DMT produced dose response disruption of a well-trained conditioned avoidance response in the shuttlebox. The criteria we tested were alterations of the drug induced behaviors by acute or chronic neuroleptic pretreatment and tolerance development. Neuroleptics, which are efficacious in the treatment of schizophrenia, reduced DMT disruption. In fact, disruption was attenuated to a degree directly related to length of neuroleptic pretreatment, consistent with long-term antipsychotic efficacy of neuroleptics. Pretreatment with dopamine antagonists and agonists suggests that the mechanism involved in DMT blockade may be through denervation supersensitivity of dopamine receptors. Complete tolerance did not develop to DMT disruption consistent with the chronic course of schizophrenia. The drug induced behavioral model reported here is of particular interest because it involves an endogenous compound implicated in the pathogenesis of schizophrenia and there is some degree of pharmacological isomorphism between this model and the clinical condition of schizophrenia.

b. Clinical

The use of GLC-MS in psychiatric disorders is particularly important as endogenous compounds of interest are frequently present in low concentrations and specificity is of considerable concern. This is particularly true for dimethyl tryptamine (DMT) and its potential role in schizophrenia. The indoleamine DMT is a known psycotomimetic agent, and has been reported to be present in human blood and urine. Moreover, an enzyme system capable of forming DMT has been demonstrated to be present in man.

Using this assay, we found no differences in DMT plasma concentrations between groups of normals, chronic schizophrenics, acute schizophrenics, and psychotic depressives. In fact, we were unable to detect DMT in plasma at all except in one normal and one psychotic depresssive. Kaplan et al. observed that DMT blood concentrations following administration of a psychoactive DMT dose (0.7 mg/kg) to human subjects were low, peaking at less than 100 ng/ml at ten minutes post-dose (the time of the greatest subjective response) and dropping off rapidly thereafter.

One possible explanation for the failure to find a correlation between blood concentrations of DMT and schizophrenia may be that endogenous DMT concentrations are exceedingly small. Any differences in DMT concentrations between normals and

schizophrenics would be missed if the higher level were still below the detection limit of the assay employed. Several refinements in the original assay procedure improved the detection limit to 0.05 ng/ml whole blood. In this study 50% of the patients and 40% of the controls gave measureable DMT concentrations. In the work described here, the assay procedure has been modified extensively, resulting in a practical sensitivity limit for DMT of 10 pg/ml whole blood.

3. Formation of Tryptolines (Tetrahydro-β-Carbolines) by Human Brain and Platelets: We have examined the products formed from tryptamine, N-methyltrypamine, and 5-hydroxytrypamine by an enzymatic preparation from either human brain or platelets, using 5-MTHF as a cofactor. In each case, we have demonstrated by TLC, co-crystallation, and gas chromatography/mass spectrometry that the reaction involves the addition of a carbon unit to the side-chain nitrogen, with subsequent cyclization to yield a tricyclic, tryptoline derivatives. These compounds have been found in plants, but have not previously been associated with mammalian systems. For both enzymatic preparations, the optimal pH is 6.5 and phosphate buffers of increasing strength enhance the reaction. For the brain enzyme, methyl-cobalamine, flavin adenine dinucleotide, and ascorbic acid each enhance the reaction, while cupric ions inhibits it. We have also done preliminary investigations on platelet activities in normal volunteers. We found marked diurnal changes and wide variability among individuals; however, male enzymatic activities were significantly lower than those for females. These studies demonstrate that 5-MTHF does not act as a methyl donor, as previously believed.

We have examined the properties and distribution of the enzymatic reaction in the human brain, using 5-MTHF as cofactor. With indoleamines, activity increases with either hydroxy or methyl substitution at the 5-position of the aromatic ring and decreases witlh the addition of methyl groups to the side-chain nitrogen. Both tryptopline and 5-hydroxytryptoline are substrates but 1-methyltryptoline is not. The enzyme has a similar Km for tryptamine and N-methyltryptamine and is inhibited by Cu^{++}, Ca^{++} and dithiothreitol. The enzymatic activity is unevenly distributed in the brain, with the highest activity in the hypothalamus and the lowest activity in the thalamus.

Acute ip injections of 5-hydroxytryptoline (5-HTLN) killed mice and rats between 12 and 48 hours post-injection with an LD_{50} dose of 320 to 350 mg/kg. Two weeks of daily injections of 200 mg/kg or 100 ng/kg 5-HTLN resulted in respective mortalities of 100% and 50%; surviving mice showed no changes in weight and gross appearance of brain, liver, lung, heart, spleen, kidney or adrenal. Neither acute nor chronic injections affected body weight or temperature except immediately before death where both dropped markedly.

In mice, one hour pretreatment with 200 mg/kg 5-HTLN ip, increased secobarbitol-induced "sleep time" (50 mg/kg, ip). At these doses, neither drug, alone, is lethal; yet, 50% of the animals treated with both drugs died 24-36 hours later. Intraventricularly, 10 µg of 5HTLN to rats decreased motor activity for 25 minutes following the injection.

Acute application of 150-300 µg of 5-HTLN to the isolated rat fundus strip contracts the muscle in a manner similar to 2-4 ng of 5-HT. When added to the

superfusion medium, 1.0 µg of 5-HTLN causes a tonic increase in muscle tone without blocking 5-HT induced contractions. Methysergide blocks these 5-HTLN effects. Preliminary studies suggest that 5-HTLN may act on serotonergic mechanism in the brain.

A case of folate-responsive psychosis which was associated with a defect of N^5, 10-methylenetetrahydrofolate reductase (methylene reductase) suggested the need to examine whether abnormally low activity of this enzyme might be of etiological significance in schizophrenia. We found that there was no significant difference in the platelet methylene reductase activity of chronic schizophrenics, compared to either hospitalized or nonhospitalized, age-matched control subjects (see table). Although it is possible that a larger survey might reveal a subpopulation of schizophrenics who are characterized by abnormal methylene reductase activity, this study suggests that chronic schizophrenia is not generally associated with such changes.

IV. Catechlamines

1. The Catecholamines:

Catecholamines and related compounds have been studied in the laboratory using a variety of approaches. Direct analysis of catecholamines, their metabolites, their enzymes, and the drugs which effect them have been examined to further understand these substances. One of the principle techniques employed to measure catecholamines and their metabolites is gas chromatography mass spectrometry (GCMS). Enzymes such as MAO and DBH have been studied with improved techniques. New drugs such as AMPT and 7-hydroxy-chlorpromazine. Chlorpromazine have also been used in clinical trials with patients. Our laboratory examines the results of all these studies in order to grasp a fuller understanding of the nature of the role of catecholamine metabolism in the schizophrenic syndrome.

a. Dopamine and Norepinephrine: Catecholamines having been investigated in the laboratory in recent years include dopamine, norepinephrine and phenylethamine. Studies of dopamine and norepinephrine have involved differentiation in post-mortem brain tissue of chronic schizophrenic patients and controls. The development of specific GLC-MS assays for DA and NE have enabled the measurement of very small (picogram) quantities of these substances in discrete brain regions. Although there were no significant differences in concentration for norepinephrine and dopamine between schizophrenic patients and controls, chronic paranoid schizophrenic patients had increased NE concentrations in the nucleus accumbens relative to other patients and controls.

b. Phenylethylamine (PEA): It has been hypothesized that phenylethylamine (PEA), a naturally-occurring amine, may be of etiologic importance in some cases of schizophrenia. PEA is structurally and pharmacologically similar to amphetamine. Furthermore, normal persons who ingest amphetamines in sufficient doses may develop a symptomatology that is clinically indistinguishable from paranoid schizophrenia.

In animals PEA produces stereotypies very similar to those produced by amphetamine. Both amphetamine- and PEA-induced stereotypies in animals are blocked by neuroleptics that are also clinically useful in treating schizophrenia. The stereotypies

are blocked by doses approximately equal to the neuroleptics' relative potencies in schizophrenia.

To investigate the relationship between PEA and schizophrenia we collected 24-hour urine samples from 23 chronic schizophrenics and 23 normals. All patients received a RDC diagnosis of chronic schizophrenia and had been chronically hospitalized for at least one year without return to premorbid level of functioning. Eleven patients were subgrouped as paranoid schizophrenic and 12 as nonparanoid schizophrenic as determined by historical review, semistructured interview, and staff consensus diagnosis bsed on DSM II categories. While many patients were on phenothiazines, we found that phenothiazines did not consistently alter urinary PEA levels in eight patients studied on and off medication. The one-way analysis of variance test was significant for 24-hour PEA urinary excretion for paranoid, nonparanoid, and normal subjects ($F=6.21$, $p<.0043$, with Brown-Forsythe Unequal Variance correction $F=3.98$, $p<.04$). The 11 paranoid chronic schizophrenics' mean $\pm$ SEM PEA excretion of 18.1 $\pm$ 5.4 ng/24 hrs was significantly higher than the 23 normal controls' excretion of 5.6 $\pm$ .5 ng (two-tailed t-test for unequal variance $p<.044$). The nonparanoid subgroup (8.6 $\pm$ 2.4) was not significantly higher than the control group.

Since MAO is decreased in the platelets and lymphocytes of some chronic schizophrenics, particularly paranoid schizophrenics, it is possible that such a reduction might represent a decreased capacity to metabolize PEA and thereby lead to PEA's accumulation. The finding of increased PEA in urine of chronic schizophrenics offers some indication that PEA may be an endogenous amphetamine.

c. Phenylactic acid: A rapid, sensitive method for the quantification of free and total PAA in 10 µl of urine by mass fragmentography was developed. The method is very reproducable showing an interclass correlation of 0.97 seg at .001 on nine samples done three times. Total PAA was determined in 12 and 24-hour collections stored at -40°C. All determinations were done in duplicate (410 determinations, interclass correlation .92-.97 on duplicates).

The mean $\pm$ SEM excretion of total, conjugated and free PAA in urine of twenty-four normal adult volunteers was respectively 137.4 $\pm$ 15.8, 128.8 $\pm$ 15.0, 8.49 $\pm$ 0.99 mg/24 hr. Free PAA constitutes less than 10% of the total (free plus conjugated) urine excretion of PAA.

The schizophrenic patients had a significantly lower ($+=2.05$, $p<.05$) urinary PAA than normals (schizophrenics 95.5 $\pm$ 15.5 mg, N=24, and normals 146.2 $\pm$ 19.7, N=22). Eight patients consistently showed extremely low PAA excretion, and only three normals showed low excretion.

Using the matched samples test, no significant difference in PAA was found between the 12-hour day urine collections (9:00 a.m. - 9:00 p.m.) and the 12-hour night collections in normals ($t=1.0$). However, the day urine collections in 12 patients were significantly lower in PAA than the night collections ($t=2.44$, $p<.05$). No significant difference was found in urine PAA before and after ingestion of ten ounces of chocolate (0.19 mg PEA) in five normals but headaches were precipitated in three.

To further explore our interest in phenylethylamine metabolism and psychiatric disorders, urines were collected from 9:00 a.m. to 9:00 p.m. on one day and 9:00 p.m. to 9:00 a.m. the following day from 13 normal adult volunteers working at the National Institute of Mental Health. After collection, the urines were mixed with 10 ml 10% EDTA, the volume measured and aliquots frozen at -40°C until analyzed. These urines were analyzed for total PAA. Twenty-four hour urines were also collected from 24 normal subjects and analyzed for free, total and conjugated PAA. The addition of 10% EDTA did not alter the urine pH. All urines were individually checked and found to have pH values between five and seven within a mean of around six. Storage of urine at -40°C did not appear to effect free PAA nor PAG concentrations. Analysis of some urines repeated about six months apart gave essentially the same results.

To test the reliability of the assay, samples from ten individuals were aliquoted into two sets of vials and blindly analyzed one week apart. The intraclass correlations for total, conjugated and free PAA were 0.98, 0.85 and 0.95 respectively. On the other hand, duplicate analysis of several urines carried out in the same batch showed correlation coefficients well over 0.95. The method described here is currently being used to evaluate PAA excretion in mental illnesses and is linear for all values so far examined. We have recently completed the analysis of urines obtained from patients on a variety of antipsychotic drugs and failed to observe a single sample with peaks that emerge near or close to those corresponding to PAA and PAA-d7.

We also examined PEA in animal experiments. Six individually caged juvenile Macaca mulatta (Rhesus monkeys) were studied during two sessions before and four sessions after IM injections of PEA. During each session, two observers who were blind to treatment conditions, counted checking (a stereotyped form of hyperigilance manifested by abrupt changes in visual field) and 27 other discrete categories of behavior. Differences between pre-drug and post-drug behavioral counts were computed for each animal.

Single injections of PEA produced dose-related (25-75 mg/kg) significant increases in checking and lip smacking (another stereotyped behavior) as well as decreases in goal-directed behaviors such as grooming. These effects, which are often accompanied by variable increases in other stereotyped behavior such as rocking, chewing, gnashing and repetitive pacing were maximal at 15 minutes post injection, still substantial at 30 minutes, and mostly dissipated by one hour. These effects are similar to those induced by the chronic administration of amphetamines. Of interest is that unlike amphetamines, consistent tolerance did not invariably develop to the repetitive administration of PEA (75 mg/kg t.i.d.) for four or nine days.

Pretreatment with alpha-methyl-para-tyrosine (AMPT) (125 mg/kg), but not with phenobarbital (25 mg/kg) substantially reduced the characteristic effects of PEA. However, when the same AMPT pretreatment was followed by a subthreshold dose of L-DOPA (5 mg/kg) typical PEA effects were restored. This finding suggests that at least some PEA effects require intact dopaminergic mechanisms and that AMPT does not block PEA receptors.

In a present study, we demonstrated that pretreatment with either a Type A MAOI, clorgyline, or a Type B MAOI, pargyline, markedly reduced the dose of PEA required to produce checking and a variety of other behavioral changes. The present

results indicate that pretreatment with MAO inhibitors, clorgyline and pargyline, potentiate the effects of PEA on checking. That is, doses of PEA (5 or 10 mg/kg), which we previously showed to be without effect, now produced significant checking values in pretreated aniamls. Neither inhibitor by itself produced checking.

Although PEA is a better substrate for MAO Type B than for Type A, both clorgyline (a Type A inhibitor) and pargyline (a Type B inhibitor) appeared to potentiate the behavioral effects of exogenously administered PEA on checking. The reasons for these observations are not fully known at this time. Previous studies have suggested that PEA releases dopamine: i.e., the behavioral effects of PEA were blocked not only by pretreatment with alpha-methyl-para-tyrosine (AMPT), an inhibitor of tyrosine hydroxylase, but by pretreatment with dopamine receptor blockers such as chlorpromazine, pimozide and haloperidol. Since both Type A and Type B MAO inhibitors may inhibit the metabolism of dopamine, the present results are compatable with the hypothesis that PEA's effects are mediated by dopamine. Further studies would be required to substantiate this hypothesis.

2. The Metabolites: A number of catecholamine metabolites and related substances have been studies using GCMS. These include DOPAC, HVA, MHPG, tyramine and octapmine. These metabolites have been studied in rats, post-mortem human brain, human CSF and plasma.

a. In rats: P-hydroxyphenylacetic, p-hydroxymandelic, 3,4-dihydroxyphenylacetic, vailmandelic acids and 3-methoxy-4-hydroxyphenylglycol were measured in controls, acutely treated and ethanol-dependent rats.

Ethanol did not cause a shift from an oxidative to a reductive pathway in the metabolism of amines as is well established in the periphery. Ethanol was found to have no effect on the transport of the acidic metabolites across the blood brain barrier.

Metabolites derived from dopamine and norepinephrine were significantly elevated in both acutely and chronically treated rats while blood ethanol levels were high. During withdrawal (no ethanol was present in blood) only norepinephrine metabolites remained elevated in rats rendered dependent on ethanol.

The concentration of VMA and MHPG in whole rat brain is 11 and 533 pmol/g, respectively. Both compounds were found in all areas of brain studied with VMA, as a percentage of both metabolites, ranging between about one and eight percent. From the decline of the compounds after pargyline, 75 mg/kg ip, we calculated that the rate of formation of VMA is 15 and for MHPG 202 pmol/g/hr. The fractional rate of elimination of VMA and MHPG is 1.4 and 0.38 hr-1, respectively. The rapid rate of loss of VMA suggests that it is transported from brain. However, we were unable to block the elimination of VMA from brain by treatment with probenecid. In contrast, the elimination of MHPG could be blocked by treatment with probenecid. Our study adds support to the notion that MHPG is a major whereas VMA is a minor product of norepinephrine metabolism in brain.

b. In Post-Mortem Human Brain: Using post-mortem brain tissue, MHPG, DOPAC and HVA were measured in chronic schizophrenic patients and controls. Free

MHPG concentrations were increased in the hypothalamus of chronic schizophrenic patients relative to controls. No such increases were seen with DOPAC and HVA.

c. Human Plasma and CSF: The concentration of the free, glucuronide and the sulfate conjugated forms of 3-methoxy-4-hydroxyphenylgycol (MHPG) were measured in human plasma, cerebrospinal fluid (CSF) and brain by mass fragmentography.

All three forms of MHPG were detected in the media analyzed. Free MHPG was found to be the predominant form in both the brain and the CSF. The sulfate conjugate of MHPG constitutes about 15% of the total MHPG in the CSF while in the brain the percentage varies between 30% in the hypothalamus and cortex and 80% in the substantia nigra.

The concentration of the glucuronide conjugate of MHPG measured in the brain and CSF represents about 5% of the total MHPG concentration.

In the plasma free MHPG and its glucuronide and sulfate, conjugates are present in about equal concentrations.

The relative concentrations of the three forms of MHPG measured in plasma, CSF and brain were compared with their concentrations in the urine from previously published results. From this comparison the diagnostic significance of each of the three forms of MHPG in the clinical assessment of central norepinephrine metabolism is discussed.

Human plasma concentrations of the major acidic metabolites of m- and p-tyramine, octopamine, dopamine, epinephrine and norepinephrine were measured. The free and conjugated forms of 3-methoxy-4-hydroxyphenylglycol (MHPG) were also measured. The means and ranges of concentrations of the acid metabolites were comparable except for m-hydroxyphenylacetic acid. Free, sulfate, and glucuronide conjugated MHPG were also found in about equal concentrations.

In a collaborative study with Dr. Norton Neff of the Laboratory of Preclinical Pharmacology, dopamine metabolism was studied in various regions of rat brain following the decline of 3, 4-hydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic (HVA) from brain after treatment with pargyline, or from the accumulation of the acids after treatment with probenecid. The decline of DOPAC and HVA after pargyline treatment appeared exponential in all regions of brain studied with half-lives of about 13 min for HVA and 6.5 min for DOPAC. DOPAC was the major metabolite of dopamine, with various brain regions producing between two to five times more DOPAC than HVA. HVA accumulated after treatment with probenecid but the accumulation in one hour did not account for all of the HVA apparently eliminated from brain. DOPAC accumulated in some regions of brain (medulla, hypothalamus, and midbrain) and not in others (cerebellum, cortex, striatum, and hippocampus) after probenecid treatment. We conclude that dopamine metabolism is not uniform in brain and that the accumulation of DOPAC and HVA in brain after probenecid treatment only accounts for a minor action of the dopamine formed in brain.

Also in collaboration with the Laboratory of Preclinical Pharmacology a study was undertaken to measure phenylalanine hydroxylase activity in vivo by measuring ^{18}O-tyrosine in plasma after exposing rats to ^{18}O. We were able to show that ^{18}O is incorporated in tyrosine in vivo and that the presence of ^{18}O-tyrosine in plasma is a relative measure of phenylalanine hydroxylase activity. Treatment with p-chlorophenylalanine decreases the ^{18}O incorporation into tyrosine. Because of the simplicity of the procedure and its easy adaptability to human studies, the incorporation of ^{18}O into endogenous constituents might serve as a useful diagnostic procedure for some metabolic disorders, for example, phenyketonuria. These results also indicate that labelling with ^{18}O cannot be used to measure the turnover rate of brain catecholamines as previously proposed.

In nine patients with ventricular-atrial shunts (5 males, 4 females, age: infancy to 65), ventricular metabolite concentrations (ng/ml, mean ± S.E.M.) were: p-hydroxyphenylacetic acid (22.4 ± 3.6), p-hydroxymandelic acid (7.1 ± 1), vanillylmandelic acid (3.1 ± 0.3), dihydroxyphenylacetic acid (9.7 ± 1.6), homovanillic acid (154 ± 14.4), free 3-methoxy-4-hydroxyphenylglycol (MHPG) (11.7 ± 1), conjugated MHPG (4.6 ± 1.3), free p-hydroxyphenylethanol (PHPE) (12.3 ± 1.7) conjugated PHPE (less than 2 ng/ml). The levels of the acidic metabolites in the lumbar CSF were all significantly lower than those in the ventricular CSF. The alcoholic metabolites on the other hand were not significantly different. P-hydroxyphenylglycol was also semi-quantitatively measured and found to be present almost completely in the free form.

Lumbar cerebrospinal fluid (CSF) of schizophrenic patients was studied.

Treatment with oral probenecid (100 mg/kg) increased the concentrations of p-hydroxyphenylacetic, 3,4-dihydroxyphenylacetic acid and homovanillic acid by over three-folds compared to the baseline levels. The concentration of p-hydroxymandelic acid was also increased, but only moderately compared to the increase of homovanillic acid. Vanilmandelic acid showed a tendency towards being elevated by probenecid, but in our study, the elevation was not statistically significant, probably due to the large variance between subjects.

3. The Enzymes

a. Brain Dopamine-β-Hydroxylase: Stein and Wise (1971) postulated that there might be a deficit in dopamine-β-hydroxylase (DBH), the enzyme that converts dopamine to norepinephrine, in schizophrenic patients.

Wise, Baden and Stein (1974) tested their hypothesis by collecting the brains of 18 chronic schizophrenics who had died in a state hospital and 12 persons without a history of psychiatric illness dying suddenly of heart attacks or accidents. The schizophrenics had decreased DBH activity ranging from 49 in the hippocampus, 58 in the diencephalon and 70 percent in pons-medulla. All these differences were statistically significant at $p < .05$. Because of these dramatic differences in the predicted direction, Wise et al. made a number of attempts to control for artifacts. After considering all these sources of artifacts, the large differences between schizophrenic and normal DBH activity remained.

We attempted to replicate their study. Specimens of autopsied brains from five chronic schizophrenic patients were obtained with the aid of the neuropathologist at Saint Elizabeths Hospital. Brain specimens from four chronic schizophrenics and nine controls were obtained by similar arrangement with the D.C. Medical Examiner's Office. Information about the subjects was obtained by interviews with family members, and hospital and police records. The control brains were obtained from periods without evidence of a psychiatric history, although one was from an individual with a police record and two were from heavy drinkers. The schizophrenics (7 males; 2 females) had a mean age of 49.2 $\pm$ 6.3. Four died suddenly after traumatic suicides (in our experience, suicide is common among chronic schizophrenics), three from cardiac arrests, one from pulmonary aspiration, and one suddenly from pulmonary edema. The controls (all male) had a mean age of 42.3 $\pm$ 4.5. Six died suddenly of trauma and three from cardiac arrests.

The periaqueductal pons-mesencephalon, hypothalamus and hippocampus were dissected out at autopsy and immediately placed on dry ice and subsequently stored at -80°C for up to one year. Wise et al. (1974) found DBH activity stable for one year at -15°C. There was no difference in mean storage time for the schizophrenics and controls. All DBH assays were performed by a staff member unaware of whether samples were from schizophrenics or controls.

There was no significant difference in DBH activity in any area between the two groups. While the differences were not statistically different, DBH activity mean for the schizophrenic groups ranged between 77 and 89 percent of the controls. Although seven of nine of our patients were taking phenothiazines at the time of death, Wise et al. (1974) indicate that this is probably not a cause of their DBH differences. Rats who had been given chlorpromazine (20 mg/kg 24 hour) for 12 weeks had a small increase in DBH activity. Although the patients in our study were taking a number of non-neuroleptic drugs, there was significant ($p < 0.05$) negative correlations between the daily dosage of chlorpromazine or chlorpromazine equivalent and the DBH activity in the hypothalamus (r=-.60) and pons (r=-.65). This could mean that in the brains of schizophrenics, neuroleptics do decrease DBH activity, or possibly that there is a negative correlation between clinical pathology (as determined by the need for higher dosages) and DBH activity.

Wise et al. (1974) also attempted to determine the effects of post-mortem intervals on brain DBH activity (after a person dies, it may take several hours for that person to reach the morgue). Simulating this situation, rats that were killed and allowed to remain three hours at room temperature prior to assay had DBH activity reduced by 27%. In a similar experiment performed in our Laboratory over a six-hour period, there was 15% decrease in activity. Since Wise et al. (1974) human brain post-mortem intervals were "several hours" and ours a mean of 4.2 for the controls and 3.0 hours for the schizophrenics, this does not seem to be an important factor, except possibly for one control who was not brought to the morgue for 27 hours. Since he had the lowest DBH activity of any of the subjects, the DBH activities were examined for the controls with and without this subject's values. The exclusion of this value did not change the failure to reach statistical significance. Two subjects with death-to-morgue times of seven and 11 hours had DBH activities about their group means. One subject with a 3.5 hour time lag was low. All other subjects had intervals of about one hour.

Wise et al. (1974) also compared the effects on brain DBH activity of the time from which the subject reached the morgue to the time of autopsy. They did this by storage of rat brains at 4°C for up to three days and found a decrease in DBH activity of 18%. Their subjects' brains, however, ranged from one day to eight days at this temperature. Because of this, they matched time intervals for controls and schizophrenics and still found the DBH activity of the schizophrenics lower. Our controls were in the morgue for a mean of 9.7 $\pm$ 1.9 hours, while the patients were there for 35.6 $\pm$ 7.9 hours, which is statistically significant ($t=2.73$, $p<.02$). Furthermore, there are significant negative correlations between the time in the morgue to autopsy and DBH activity in the hippocampus ($r=-.53$, $p<.05$) and hypothalamus ($r=-.49$, $p<.05$) while that for the pons ($r=-.46$) was not significant. This suggests that the uneven distribution between the two groups in morgue time may be responsible for the small non-significant differences in DBH activity in our samples. Since the Wise, Baden and Stein brains came from two sources, it is possible some of their group differences may be due to different storage technique.

We measured DBH activity both under maximal conditions as well as under non-maximal conditions, but did not find significant differences among patient groups and normal controls by enzyme procedure.

Differences in population characteristics such as race and sex are suggested by the trend in blacks to have lower DBH activities and females to have higher activities than males. The relationship of age to DBH enzyme activity is not known but may also be a contributing factor and should be further explored.

Previous reports have not determined DBH activity in various subtypes of schizophrenia. Although we found no significant differences among the subtypes and normals, the chronic undifferentiated group with paranoid features had a trend toward lowered DBH activity ($p<.2$ and $p<.13$). Further investigation of this subtype appears warrented.

One way analysis of variance shows no significant difference between control and schizophrenic subpopulations. The chronic undifferentiated schizophrenics with paranoid features had a somewhat lower mean DBH than the other patient groups or normal controls, although this does not reach statistical significance. The normal control group had a mean DBH of 37.28 $\pm$ 26.00.

In addition, DBH and monoamine oxidase activities were studied in 13 schizophrenic patients and available first degree relatives. There was no association of low monoamine oxidase and low DBH activities within the schizophrenic families.

In summary, in our investigations, we have been unable to confirm the findings of low plasma DBH activity in chronic schizophrenic patients or the association of low DBH and MAO activity in pedigrees of schizophrenic probands.

In addition, we have found that DBH activity is decreased by neuroleptic medication. Since most studies have been done in patients on chronic medication treatment (either before or after death) a drug effect could explain these findings.

b. Dopamine Sensitive Adenyl Cyclase: Striatal adenyl cyclase activity from autopsied human brain is selectively stimulated by low concentrations of dopamine. Under the experimental conditions used, dopamine was about three times more potent than norepinephrine. Histamine and serotonin were ineffective. There was no difference between nine competitively inhibited by haloperidol. There was no difference between nine of the autopsied brains (head of caudate) of the control subjects without a psychiatric history and seven patients with chronic schizophrenia.

c. MAO: The laboratory has devoted considerable time and attention to exploring the role of MAO in the schizophrenic syndrome. The finding that blood platelet monoamine oxidase (MAO) activity is low in some schizophrenic patients has led us and others to inquire as to its significance. As part of this endeavor, there has been a continuous effort to determine if this low enzyme activity was due to one of the large number of artifacts that have plagued the investigation of biological factors related to psychiatric disorders.

1. MAO Assay in Blood Platelets: We are currently employing a new method for the preparation of platelet pellets, developed by Corash and Shafer. This procedure gives 98 percent recovery of the platelet population with no plasma protein contamination and with virtually no red blood cells or leukocyte contamination. Every aspect of this detailed procedure has been carefully designed so that the platelets remain physiologically intact and do not clump and trap plasma proteins.

2. Chronic Schizophrenics: To date we have studied platelet MAO activity in 104 male chronic schizophrenic patients. Their mean $\pm$ S.E. MAO activity was 7.86 $\pm$ .56 while the value for 348 normal males (ages 18-50) was 11.04 $\pm$ .29. This difference using a two-tailed "t" test, was significant at $p < .001$. The mean for the 15 female chronic schizophrenics was 7.04 $\pm$ 1.06 while that for 332 normal females was 13.29 $\pm$ 0.29 ($p < .001$).

The effect of chronic hospitalization on platelet MAO was studied at the Palo Alto VA Hospital where 50 normal controls were compared to 16 patients hospitalized for traumatic accidents to their spines. There was no difference between MAO activity between groups but 39 chronic schizophrenics had decreased platelet MAO activity.

At least 26 studies have been published concerning the enzyme, monoamine oxidase (MAO), in platelets and white cells of chronic schizophrenic patients. Although at times the deviation from controls has been very small, in all but four instances a decrease in MAO activity has been found. In 19 studies, the reported decrease was statistically significant. Using the sign test, the chance of 22 of 26 studies demonstrating a decrease is less than 1 in 4,000 and the possibility of 19 of 26 being statistically significant at the 0.05 level by chance alone is less than 1 in 10 (binomial test). In spite of this evidence, the finding continues to be thought of as controversial. We believe that what is controversial about the finding is not whether or not platelet MAO activity is decreased in some schizophrenics, but 1) to what degree, 2) in which patients, and 3) what, if any, biological significance this decrease might have.

3. Acute vs. Chronic Schizophrenics: To determine whether acute schizophrenics have a reduction in platelet MAO as did the chronic schizophrenics, platelet MAO of 27 acute schizophrenics were studied. The ten male acute schizophrenics (ages 16-30) had a mean platelet MAO activity of 10.68 $\pm$ 78 which was no different than that of normal males. The 17 female acute patients had a mean of 13.85 $\pm$ 1.12 which was no different from that for the normal females.

4. Subclass of Schizophrenia: In 1974-1975 we undertook a retrospective study of 43 patients we had examined to look at who the patients were with low platelet MAO. Information was obtained regarding circumstances and age at onset of illness, presenting problems, diagnoses and family history of mental illness. The patient's medical and surgical history was reviewed, as well as laboratory data which included EEG's and skull x-rays. Mental status examination on admission and throughout hospitalization was summarized, as well as changes observed relating to medication. This information was gathered and coded without knowledge of platelet MAO activity.

Two types of statistical analyses were employed. The first approach correlated MAO activity with a variety of clinical variables using the Pearson product-moment correlation coefficient. The second approach looked for differences between groups of patients with low and high MAO activity. In the second approach, the MAO value of 9.16 units was arbitrarily chosen to divide the chronic schizophrenic patients into high and low MAO groups (12 patients in the high group; 31 patients in the low group). 9.16 is two standard errors above the female chronic schizophrenic mean (8.04 and slightly higher than two standard errors above the male schizophrenic mean (7.86). The term "high MAO" in this study refers to high MAO activity for chronic schizophrenic patients. The high MAO group has a mean $\pm$ SEM of 13.54 $\pm$ 1.06, which is comparable to values for the normal population. The low MAO group had a mean of 5.47 $\pm$ .37. All patients were diagnosed chronic schizophrenics with a mean hospital stay of $\bar{9}$.3 years, although length of hospitalization within the sample did not correlate with MAO activity (r=.11). MAO activity did not correlate with age at onset of symptoms (r=.06), age at first hospitalization (r=.12), length of hospitalization (r=.11), or with problems at work (r=.20), school (r=.07), or marriage (r=.12).

Schizophrenic patients with low MAO had a history of more seizures (r=.31, $p < .05$) than patients with high MAO. Of the 41 patients from whom we had an adequate history of seizures, eight of 30 low MAO schizophrenics had at least one seizure during hospitalization, compared to one of 11 high MAO schizophrenics. The high-low difference was not significant using the Fisher Exact Probability Test. None of the patients was on anticonvulsive medication. The possibility of abnormal brain dysfunction correlating with low MAO was reflected in the significant negative correlation between MAO and the number of abnormal EEG's (r=.32, $p < .05$). Of those 36 patients who had EEG records available, 10 of the 25 low MAO schizophrenics had at least one abnormal EEG, compared to none of the 11 hight MAO schizophrenics (Fisher Exact Probability Test, $p < .01$). The data indicate that low MAO activity in chronic schizophrenics is associated with an increase in abnormal EEG's and perhaps an increase in seizures. This abnormal brain activity is, however, not associated with other medical or asurgical problems, or with any abnormalities in routine blood studies.

In regard to the mental status examination, the patients with low MAO activity were reported to have more ambivalence ($r=.32$, $p<.05$) but not any difference in severity of frequency of thought disorder ($r=.02$), associational defects ($r=.10$), autistic behavior ($r=.03$), hallucinations ($r=.09$), instances of self-mutilation ($r=.04$) or suicide attempts ($r=.24$). No evidence for differences in treatment response related to MAO activity was found ($r=.05$). Mental illness in relatives did not correlate ($r=.19$) with platelet MAO activity and was not different betweeen the high and low MAO groups. However, it might be impossible to determine such a correlation from the relatively small sample. This is compounded by the inherent difficulties in determining a complete accounting of family incidence of mental illness from chart analysis.

All of the paranoid schizophrenics had low MAO. None of the high MAO activity schizophrenics received a DMS II diagnosis of paranoid schizophrenia during their hospital stay; seven of the 30 low MAO group did (Fisher Exact Probability Test $p<.05$). This difference was further substantiated when the means of MAO activity were compared between those who received a diagnosis of chronic paranoid schizophrenia and those who did not. The paranoid schizophrenics have a mean MAO activity of $4.8 \pm .46$, which is significantly lower than the nonparanoid schizophrenic values of $8.6 \pm .75$ (t-test, $p<.03$). This difference was also reflected in symptoms characteristic of chronic paranoid schizophrenia, such as paranoid delusions and increased hostility, but not in other less specific symptoms.

From this study emerged a picture of a low platelet MAO activity schizophrenic who is more frequently diagnosed as paranoid, as having more paranoid type delusions, and as having abnormal brain activity reflected by increased incidence of seizures and abnormal EEG's.

In order to further examine these findings, 42 new patients were selected on the basis of definite diagnosis of schizophrenia who had active symptoms but without regard to subtype. Patients with a diagnosis of organic brain syndrome or mental retardation of other cerebral pathology, including severe alcoholism, were excluded in order to focus on the paranoid dimensions. The 42 subjects were drawn from a sample of 65. Fifteen were excluded because of their history, eight others met criteria but refused to take part in the study. Seven of these eight refused because of apparent suspiciousness or distrust of the experimental procedure.

All patients met Research Diagnostic Criteria and 41 of 42 met Feighner's criteria for a diagnosis of schizophrenia. The mean age was 35, mean length of hospitalization of 401 months, and all patients had, at one time, received phenothiazine treatment. All patients had a chronic course and 40 of the 42 had a gradual or insidious onset of psychosis. Twenty-nine were males and 13 females. MAO values were 6.7 and 10.6 units for males and females, respectively, compared to mean MAO's of 11.04 and 13.29 for normal males and females. The difference between the schizophrenics and normals was significant at $p<.01$. The clinical staff who best knew the patient rendered a consensus diagnosis based on DSM II categories. A secondary diagnosis was reached for cases of chronic, undifferentiated schizophrenia. All patients underwent a semi-structured interview. Interviews were conducted at a time as close as possible to blood drawings, and for most patients were done on one occasion over a 60-minute period. Repeated interviews were occasionally required to obtain the necessary information.

The information was gathered and coded without knowledge of MAO values. The data indicate that patients diagnosed as paranoid schizophrenics had a mean platelet activity MAO level of 5.4 for males and 8.9 for females, compared to 8.6 and 12.1 for nonparanoid male and female schizophrenics. Patients with secondary paranoid features had MAO values of 5.6 for males and 9.1 for females. Values for both paranoid patients and patients with secondary paranoid features were significantly different from those of nonparanoid patients ($p < .01$).

The variables which best diagnostically divided the paranoid from nonparanoid patients by a linear discriminate function were: degree of suspiciousness, preoccupation with and systematization of delusions, delusions of control, stereotyped behavior, intact memory, rigidity of ideas, and communication characterized by evasiveness and misleading responses. These diagnostically discriminating items are similar to items on the Venables Scale for Paranoid Schizophrenics except for grandiosity which was not a good discriminator in our study.

These prospective findings confirm the retrospective chart analyses. Chronic schizophrenics had lower platelet MAO activity than normals. Paranoid schizophrenics had an even lower MAO activity level, whether the paranoia was determined by clinical diagnosis, by interview, or by a paranoia dimension scale.

5. Specificity with Regard to Other Illness: Since the various psychiatric diagnostic classifications are thought to represent different entities, a defect in one should not be seen in the order. The affective illnesses are the group we most diligently contrasted with the schizophrenics.

Sixteen male and 13 female unipolar depressed patients were found to have platelet MAO activity that was no different from our controls while 16 male and 33 female bipolar I depressed patients had reduced activity ($p < .001$). The bipolar patients had a negative skew which was not as large as that of the chronic schizophrenics. Using six units of MAO for the male normals and eight units for the normal females as a point of cut-off, a point under which less than 10 percent of normals fit, 39 percent of male chronic schizophrenics and 60 percent of the female chronic schizophrenics are found (chi square $p < .001$). The bipolar patients also differed from normals ($p < .001$ males; $p < .02$ females). The male bipolar patients were not significantly different from the male chronic schizophrenic patients, but the percentage of female chronic schizophrenics with MAO activity below eight units was greater than the female bipolar I patients. Thus, although bipolar I patients using the arbitrary cut-off have a mean MAO which is low, it is not clear whether they represent a separate group from the chronic schizophrenic patients.

6. Twins Discordant for Schizophrenia: To exclude the possibility that the low platelet MAO activity seen in the chronic schizophrenics was caused by nongenetic factors as opposed to genetic ones, we studied monozygotic twins discordant for schizophrenia. If the low platelet MAO activity is due to some aspect of being schizophrenic, low platelet MAO should only be present in the schizophrenic twin. Thirteen schizophrenic index twins, all of whom had been hospitalized at least once for schizophrenia and had been extensively studied by Dr. William Pollin and his associates, were examined along with their non schizophrenic co-twins. At the time of the study, one patient resided within a hospital while five were in remission. Four of

the patients had acute forms of schizophrenia and six patients were not receiving any antipsychotic medication. The nonschizophrenic co-twins had never been hospitalized for a behavioral disorder and were functioning well within their families and communities, except for one individual with borderline psychosocial adjustment.

The MAO activity of the 23 normal controls was the same as that obtained at the time for previous controls (16.02). The schizophrenic twins (9.88, $p < .005$) and nonschizophrenic twins (12.08, $p < .05$) were significantly lower than the normals, but there was no difference between the twin groups. There was a significant Pearson correlation ($r = .67$, $p < .01$) between the MAO activities in the schizophrenic and nonschizophrenic twins.

Platelet MAO activity was also examined in nine monozygotic and 10 dizygotic normal twins. The intraclass correlation coefficient was 0.88 for the monozygotic and 0.45 for the dizygotic twins. The correlations were significantly different from one another (Mann Whitney U; $p < .001$). Same-sex siblings matched for age and sex were about the same as the dizygotic twins while unrelated similar to those reported by Nies, Robinson, Lamborn and Lampert (1973) for normal monozygotic and dizygotic twins using benzylamine instead of tryptamine as a substrate.

Taken together this data indicates that platelet MAO activity is in a large part determined by genetic factors.

7. Family Studies: In order to further study the relationship of low platelet MAO to schizophrenia the blood of first-degree nonschizophrenic relatives of index schizophrenics (N=16) was collected. Dividing schizophrenic indexes at their median, the low indexes had a mean of 4.64 and the high indexes had a mean of 10.81. The mean MAO activity for the low index first-degree relative was 8.56. The mean activity for high index first-degress relatives was 11.84. The difference betweeen the means for the low vs. high first-degree relatives was significant ($p < .02$; Mann Whitney U). No attempt was made in this small sample to account for sex differences. These differences between nonschizophrenic first-degree relatives of high and low schizophrenics is another indication of the genetic nature of platelet MAO activity.

Ongoing family studies for the past two years presently include studying MAO in platelets and lymphocytes in first-degree relatives of schizophrenics. At present, findings are inconclusive. As well, a collaborative effort is being established with other Divisions of the Hospital to study the inheritance of schizophrenia by hospitalizing first-degree relatives with schizophrenia.

8. Physical-Chemical Studies: In one study, we determined the rate of heat inactivation of the MAO in a group of chronic schizophrenic patients (N=22) and normal subjects (N=13). Aliquots of platelet MAO were subjected to different temperatures between 56°C and 62°C for five minutes. Analysis of the data revealed no significant differences in the rate of heat inactivation between the normal and schizophrenic groups confirming an earlier study from this laboratory. However, when the schizophrenic group was divided into paranoid schizophrenic and chronic undifferentiated schizophrenic (CUS) groups, differences became apparent. The MAO in the paranoid group was most resistant to heat while the MAO in the CUS group was the least resistant; the MAO in the normal group was intermediate in its rate of heat

inactivation. There was a significant difference ($p < .05$) between the paranoid and nonparanoid (CUS) groups. Neither schizophrenic group differed significantly from the normal group. However, the combination of the normal plus the CUS group differ significantly from the paranoid group ($p < .035$).

In a second study, we have attempted to replicate a finding reported by Berrettini and Vogel that an inhibitor of platelet MAO is present in the plasma of schizophrenic patients that have low MAO activity. If their report were correct, it would provide a mechanism that would explain the reduced MAO activity found in schizophrenic patients. However, we have not been able to replicate their results. In fact, in five of six experiments we have found a slight increase in MAO activity when normal platelet MAO is preincubated with plasma from schizophrenic patients (effect of chronic plasma on Km and Vmax, 1.5 ± 0.05 and 22.1 ± 2.46; effect of schizophrenic plasma on Km and Vmax, 1.8 ± 0.28 and 24.9 ± 1.57). We would conclude from our studies that an inhibitor does not appear to be present in schizophrenic plasma.

In a third study, we are attempting to replicate the finding of a reduced Km in the platelet MAO in a group of paranoid patients compared to a group of normal subjects. Although our preliminary findings are in the right direction, certain methodological problems have become apparent and need to be resolved before we can complete the study.

If this finding can be firmly established in the schizophrenic group, we then plan to proceed and to measure the Km in a group of alcoholic patients and in a group of bipolar depressive patients. The overall purpose would be to determine if the decrease of Km of the schizophrenic patients observed is unique to that patient group or is shared by the other two patient groups that have also been reported to have a reduced platelet MAO activity.

Heat Inactivation: Most studies have reported that chronic schizophrenic patients have reduced platelet monoamine oxidase (MAO) activity. Possible environmental and genetic factors that might cause the lower activity have been subject of intense investigation. On the other hand, most environmental factors, such as drugs and hospitalization that might cause the decreased MAO activity have been excluded. On the other hand, it appears that the level of MAO activity in both normal and schizophrenics is, at least in part, due to genetic factors. Given the possibility of a genetic contribution, it seemed reasonable to determine if the MAO itself was altered. To determine this, we have examined some physical-chemical properties of MAO. We and others have shown that with some substrates the Km is altered in the MAO of chronic schizophrenic patients. In the present study, we have attempted to examined the rate of heat inactivation of platelet MAO in a group of normal subjects and chronic schizophrenic patients.

Berrettini and Vogel described evidence for an inhibitor of platelet monoamine oxidase (MAO) in the blood of chronic schizophrenic patients. They suggest that this endogenous inhibitor may be responsible for the decreased platelet MAO activity reported in chronic schizophrenics. We, however, failed to demonstrate the presence of this inhibitor.

The blood from eight patients with low MAO activity was used. The plasma from two patients was provided by Dr. Berrettini. The other six patients were hospitalized at Saint Elizabeths Hospital where they received a diagnosis of schizophrenia both by Feighner and Research Diagnostic Criteria. The patients who satisfied these criteria had a chronic illness and were unable to function in the community. Additionally, the clinical staff who best knew the six patients rendered a consensus subtype diagnosis of paranoid schizophrenia based on DSM-II categories. Since Berrettini and Vogel did not use drug free patients and since neuroleptic drugs seem to have little effect on MAO activity, the patients used in the present study were not neuroleptic free. Staff and laboratory personnel were used for the control population. The procedure for determining the presence of an inhibitor was that employed by Berrettini and Vogel. They indicate that the inhibitor is present in the platelet-poor plasma (PPP) of schizophrenic patients who have low platelet MAO activity. To briefly describe their procedure, three aliquots of platelet-rich plasma (PRP) from a normal were used in each experiment. One aliquot of the normal PRP was unmixed. The second PRP aliquot was mixed with PPP that was obtained from a second normal control which presumably contained no inhibitor. The third PRP aliquot was mixed with the schizophrenic PPP. All three samples were incubated for 10 min at 37°C. The samples were centrifuged to obtain the platelet pellet which was then washed and resuspended in buffer. The kinetic parameters (Km and Vmax) for the three samples were determined.

The enzymatic assay method of Wurtman and Axelrod was used. Radioactive tyramine (tyramine [1-^{14}C]) and radioactive dopamine (dihydroxyphenylethylamine hydrobromide, 3,4 [ethyl-^{14}C]) were obtained from New England Nuclear. When tyramine was employed as a substrate, five concentrations were used that range from 10×10^{-4} to 1×10^{-4}M. The reaction was stopped with perchloric acid and the product was extracted with toluene. When dopamine was employed as a substrate, five concentrations were used that ranged from 8×10^{-4} to 0.5×10^{-4}M. The reaction was stopped with hydrochloric acid and the product extracted with toluene: tetrahydrofuran (2:1, V:V). Protein was determined by the method of Lowry et al.

The Km's and Vmax's were obtained by fitting data with a PDP-11 computer to a rectangular hyperbola according to the iterative procedures of Bliss and James. This method yields the same results as those in procedures developed by Cleland. The data were then further analyzed with a two-tailed paired t-test.

The data indicate that there were no significant differences among any of the groups in either the Km and Vmax measurements. Berrettini and Vogel reported that treatment of normal platelets with schizophrenic platelet-poor plasma reduced both the Vmax and Km of normal MAO. We did not find reductions; if anything, our data indicate that the schizophrenic plasma produced nonsignificant increases in MAO activity. More specifically, the schizophrenic plasma produced increases in the Vmax in six out of eight cases when tyramine was used as a substrate ($p < .06$). In addition, the two schizophrenic plasmas provided by Dr. Berrettini also produced increases in the Vmax.

It is not clear why we were unable to replicate the findings of Berrettini and Vogel. If there is an inhibitor, there must be factors affecting the procedure of which Berrettini and Vogel are not aware. One possible factor involved could be the

procedure used in analyzing the data. We used the objective mathematical method of Bliss and James. Berrettini and Vogel used Linewearver-Burk plots in which the straight line was fitted by eye to obtain their Vmax and Km values. This plotting procedure has been shown to be subject to subtantial error. Dowd and Riggs conclude that, "The marked inferiority of the Linewearver-Burk plot strongly suggests that it should be abandoned as a method for estimating Km and Vmax from unweighted points, whether the points are fitted by eye or by the method of least squares." Furthermore, if Berrettini and Vogel did not plot their data as a blind procedure, an additional error could have been introduced.

9. Relationship of Platelet MAO in Brain MAO: The number of types of MAO present in the brain is an unresolved question. Original studies indicate that there might be as many as four isoenzymes of MAO within the brain; however, Houslay and Tipton (1973) suggested that the multiple enzymes found using polyacrylamide-gel electrophoresis might be produced artifactually in a preparatory step. The authors state that these multiple MAO forms may represent a single protein with varying amounts of phospholipid attached; the amount of phospholipid may, in turn, determine the enzyme mobility during electrophoresis.

Another approach to this problem has come from the finding that selective inhibition of MAO can be used to identify multiple forms of MAO. Clorgyline was the first MAO inhibitor employed in this manner. It was found that tissue homogenates pretreated with increasing concentrations of clorgyline produced stepwise inhibition to the substrate tyramine, suggesting the presence of an enzyme sensitive to the inhibitor and one relatively resistent to it. Considerably more work has been done to identify these enzymes and for convenice they are called Type A and Type B.

The preferred substrates for the Type A MAO are norepinephrine, serotonin, and normetanephrine, while for the Type B they are benzylamine and β-phenylethylamine. Dopamine, tyramine and tryptamine are common substrates. Clorgyline, Lilly 51641, and harmaline are specific inhibitors of Type A while deprenyl and pargyline inhibit Type B. Most of the other MAO inhibitors used in clinical medicine, however, are nonspecific. Platelet MAO, forming one band on electrophoresis, appears to be a single enzyme with Type B characteristics. That is, the platelet enzyme demonstrates only a single sigmoid curve for inhibition using deprenyl, pargyline, or clorgyline. Furthermore, the platelet enzyme is 200-fold more sensitive to inhibition by deprenyl than clorgyline. The conclusion that the platelet enzyme is a simple Type B MAO is also supported by the high activity of the platelet enzyme with the relatively specific MAO-B substrates, benzylamine and β-phenylethylamine, in contrast to its lesser activity with serotonin, an A substrate.

10. Function: To determine whether or not the low platelet MAO activity might have a functional significance, platelet-rich plasma was obtained from 20 normal controls (age 29.6 $\pm$ 1.1) and 16 chronic schizophrenic patients (27 $\pm$ 1.4). Since plasma serotonin is almost entirely contained in the platelet the values were expressed in platelet units (ng/10^8 platelets). The platelet counts for the normals and schizophrenics were the same. When the patients had been off phenothiazines (N=12) for 30 days or longer the serotonin concentration was higher (80 $\pm$ 4), while when the patients were taking phenothiazines (N=12) their platelet serotonin concentration was normal (90 $\pm$ 9). The reason for the discrepancy between patients taking and not taking

phenothiazines may be due to the latter's ability to block serotonin uptake into platelets. Nevertheless, since serotonin is not a good substrate for platelet MAO (Km 10^{-3}) other substrates might be much more affected.

Increases in plasma prolactin concentrations produced by alpha-methyl-para-tyrosine, a catecholamine synthesis inhibitor, varied inversely with baseline platelet monoamine oxidase activity in 12 chronic schizophrenic patients. Two subsequent studies with 39 normal volunteers and 19 unmedicated chronic schizophrenic patients were conducted to test for a relationship between platelet monoamine oxidase activity and plasma prolactin concentration. Plasma prolactin concentrations were found to vary directly with platelet monoamine oxidase activity in normals with low monoamine oxidase activity and patients with chronic schizophrenia. No relationship was found in normals with high platelet monoamine oxidase activity. These data suggest that platelet monoamine oxidase activity reflects monoaminergic activity in the tubero-infundibular system which in turn affects plasma prolactin concentrations. This relationship may be particularly important in patients with low platelet monoamine oxidase activity such as some chronic schizophrenics.

Further evidence to support the notion that platelet MAO activity may reflect MAO activity in the brain has been suggested by the finding of a reluctancy between eye blinking and platelet MAO activity. Chronic schizophrenic patients' platelet MAO activity has been found to correlate inversely with the eye blink rates, a physiological parameter which may be indicative of central nervous system dopaminergic activity.

11. Discussion: What does in mean to have low platelet MAO activity in veiw of seemingly normal brain MAO activity? The argument that post-mortem measurement of brain MAO activity does not reflect its true activity because of autolysis has little support since brain MAO seems to be a very stable enzyme. It remains, of course, a possibility that we have either not measured brain MAO activity in the right region or with correct methods, an argument that is not easily dealth with.

Enzyme activity must now be measured in other regions of the body, such as the muscle, lung, liver and kidney. If these areas turn out to have normal MAO activity while the platelet is low, intriguing explanations will be needed.

Part of the attractiveness of the concept of low MAO activity is that it is congruent with the two most generative biological hypotheses of schizophrenia. The first of these suggests that there is a functional excess of dopamine. A defect of MAO, an enzyme involved in dopamine metabolism, might be able to produce "dopamine excess." The second hypothesis suggests the formation of an hallucinogenic methylated idoleamine (transmethylation). Since the indoleamines as well as their methylated congeners are metabolized by MAO, this theory is also consistent with a finding of low MAO activity. One of many questions that needs to be answered before low MAO activity can be considered causative, is "Why don't the vast majority of people given drugs which inhibit MAO become psychotic?"

12. Lymphocyte MAO: To look at MAO activity in other tissues, we explored MAO in lymphocytes. We found lower MAO activity in schizophrenic patients than controls. There was a modest correlation between lymphocyte and platelet MAO in

normals. However, other variables (such as age, sex, race) contributed to levels of MAO activity.

Drugs Which Effect Catechols Metabolism

4. The Drugs

Pharmacological investigations of the roles of catecholamines in the schizophrenic syndrome have involved synthesis inhibitors and receptor blockades. Under the former, the synthesis of DA and NE has been inhibited by AMPT. Under the latter, DA and NE receptors have been blocked using 7-hydroxy-chlorpromazine and propranolol respectively.

a. AMPT (Alpha-Methyl-Para-Tyrosine): Several lines of evidence suggest that certain types of schizophrenia may be associated with excessive dopamine activity. Psychotic symptoms in schizophrenics may be intensified by methylphenidate, which increases functional dopamine activity, and by L-DOPA, which is converted to dopamine. Amphetamine-induced psychosis in volunteers and in "speed freaks" in thought to be mediated by dopamine. The clinical efficacies of neuroleptics depend upon blockade of dopamine receptors. Alpha-methyl-para-tyrosine (AMPT) inhibits tyrosine hydroxylase, the rate limiting enzymatic step in dopamine synthesis. Although it should theoretically be clinically helpful in schizophrenics, two previous studies reported no effect when given alone. In a third study, however, Carlsson and his associates found that during treatment with AMPT, less thioridazine was required to treat schizophrenics. In this study, we report results of administering AMPT in a double-blind fashion to chronic schizophrenics who were concurrently treated with high doses of phenothiazines.

The subjects were ten male chronic schizophrenics (age 19-35) who had been hospitalized for 2-15 years. Each received a constant dose of phenothiazines (average equivalent dose of chlorpromazine=800 mg/day). A four-week period of placebo administration preceded and followed by a three-week trial of AMPT (3 gm/day). Clinical effects were monitored by (a) the NIMH Nursing Rating Scale; (b) the BPRS administered by two psychiatrists weekly; and (c) the Comprehension Subscale of the WAIS before, during, and after AMPT administration.

Significant improvement was observed on the Comprehension Subscale ($p < .001$ compared with placebo periods) on AMPT. However, no improvement was noted in the schizophrenia items of the BPRS or the NIMH Nursing Rating Scale, except for one patient. Clinical impressions of three, nonblind psychiatrists suggested moderate but transient improvement during the first week of AMPT administration in eight of ten patients. Two patients developed symptoms on AMPT and one was withdrawn from the study.

The results suggest that during a three-week trial with AMPT, an inhibitor of dopamine synthesis, subtle or no improvement in psychological functioning (judgment and comprehension) occurred in chronic schizophrenic patients on phenothiazines. Significant clinical improvement was not detected on the BPRS and NIMH Nursing Rating Scale.

In a second study, we admnistered AMPT (3 gm/day) for two-three months to seven chronic schizophrenic patients who were treated with thioridazine (800 mg/day). No major clinical benefits were documented in the second study, although serum prolactin appears to have been elevated, an indication of central dopaminergic blockade.

b. 7-Hydroxy-Chlorpromazine: A number of biochemical, animal and human studies suggest that 7-hydroxy-chlorpromazine (7-OH CPZ) is an active metabolite of chlorpromazine (CPZ). This hypothesis was treated by administering 7-OH CPZ to four hospitalized chronic schizophrenic males (Research Diagnostic Criteria) using a double-blind crossover design.

Following a four-week placebo period, 7-OH CPZ was given for at least four weeks, three weeks of which were at 1000 mg/day. A second four-week placebo period was then followed by the administratin of CPZ at the same dose and duration as 7-OH CPZ. Throughout the study, patients were evaluated twice/day a nurse' rating scale and once/week with the BPRS. Serum prolactin levels were measured at least once/week. Data was evaluated by an analysis of variance and the Newman-Keul test.

Patients improved on 7-OH CPZ relative to placebo on the following NRS items: tension and uncooperativeness ($p < .01$); and blunted affect, hostility, excitement and arousal ($p < .05$). With regard to CPZ relative to placebo, improvement was demon strated on tension and blunted affect on the NRS ($p < .05$). 7-OH CPZ was more effective than CPZ on uncooperativeness on the NRS ($p < .01$). On the BPRS, patients improved on 7-OH CPZ relative to placebo on total score and emotional withdrawal ($p < .05$). Again, on the BPRS, CPZ was more effective than placebo on activation and anxiety-depression ($p < .05$). Finally, 7OH CPZ reduced emotional withdrawal more than CPZ ($p < .05$) as measured on the BPRS. Improvements on the NRS and the BPRS ranged from 25.5% to 50.9% for statistically significant items. Prolactin levels on 7-OH CPZ and CPZ were 33.1 $\pm$ 4.5 ng/ml and 31.1 $\pm$ 8.7 ng/ml, respectively. These were elevated by 119.2% and 84.0% over placebo levels of 15.1 $\pm$ 4.5 ng/ml and 16.9 $\pm$ 4.5 ng/ml, respectively ($p < .05$).

The above results clearly show that 7-OH CPZ is an active neuroleptic producing clinical improvement in chronic male schizophrenics. Moreover, it caused substantial increases in serum prolactin levels, a property of its metabolic precursor, CPZ and numerous other antipsychotic dopamine blocking neuroleptics.

c. Propranolol: It has been reported that high doses of β-adrenergic blocking agent propranolol can be therapeutic in schizophrenia. In an effort to confirm this finding, 11 young chronic schizophrenic patients have entered an ongoing double-blind crossover study of their clinical and metabolic response to this drug.

Subjects were patients on two research wards, who (1) were between age 18 and 45 years, (2) met Research Diagnostic Criteria for schizophrenia, (3) had adequate trials of conventional neuroleptic treatment without achieving sufficient remission to enable living outside of a hospital setting, and (4) were able to consent to participate in therapeutic trials. Patients were maintained on stable doses of neuroleptics throughout the trial. Treatment order, active-placebo or placebo-active, was determined randomly. Propranolol was given incrementally over a 5-wk period to a

maximum of 1920 mg/24 hrs in two divided doses, unless the presence of side effects dictated a lower maximum. Patients were kept at their highest stable doses for four weeks and then tapered off propranolol over a further 4-wk period. Behavioral status was evaluated by the nursing staff who were blind to medication status using a expanded version of the Brief Psychiatric Rating Scale (BPRS). Behavioral ratings were analyzed as previously described.

Of the 11 patients entered in the protocol, seven have completed a month of placebo and a month of stable dose of 1600 or 1920 mg of propranolol. One patient refused further participation after one week and a second patient had to be withdrawn because of postural hypotension. The results to date indicate the presence of modest changes in some ratings for each patient. No patient had a dramatic response, but the improvement in two patients seemed substantial to those who knew them.

Our clinical results thus far do not confirm eariler reports of marked benefit to treatment resistant patients, but the duration of treatment was longer in those trials. Further experience will be required to determine if there is a subgroup of patients who are responsive to treatment with propranolol. If such a group can be defined, it would be of great interest to discover the mechanism of action of propranolol in that group. Since propranolol is active both centrally and periph erally, and in addition has membrane stabilizing properties and antiserotonergic actions, there would be many possibilities.

GABA: Gaba is a neurotransmitter thought to be related to dopamine metabolism. To investigate this relationship we conducted the following experiments:

I. Clinical

Baclofen (Lioresal): In the spring of 1975, a brief clinical report appeared in Lancet stating that 13 treatment resistant chronic schizophrenics were found to improve and require less conventional neuroleptic medication when placed on Baclofen. Since Baclofen, then thought to be a GABA agonist but not thought to be an antagonist of substance P, is a compound in no way similar to currently used neuroleptic agents, we have conducted a clinical trial.

In an attempt to replicate claims of baclofen's efficacy in treating schizophrenia, baclofen was given to young chronic schizophrenic patients and plasma prolactin was monitored to study the mechanism of action of baclofen. Seven hospitalized male chronic schizophrenic patients were stabilized on clinically suboptimal doses of conventional neuroleptics (equivalent to 800 mg/day of chlorpromazine). Experience with these patients revealed that these doses were necessary for participation in the protocol and that larger doses resulted in less symptoms but not to the point that patients could function outside of the hospital. Baclofen was then added in doses of 80 to 120 mg/day for one month in a double-blind, crossover design. Twice weekly, fasting bloods were drawn 11 hours after the last neuroleptic and baclofen dose (into heparinized tubes which were then placed on ice). Plasma was removed and samples stored at -70^{o}C until radioimmunoassay by the double antibody technique. The prolactin values during each period were stable for each patient as reflected by an intraclass correlation of .93 for the neuroleptic period and .87 for the

baclofen period. For each patient, biweekly prolactins were meaned separately for the placebo periods and then compared by paired t-test.

No increase in plasma prolactin was found with the addition of baclofen (37.08 vs. 37.57 ng/ml, paired t-test, NS) to patients given sub-optimal doses of neuroleptics. There was no evident difference in response on two dose levels. Additionally, the patients did not clinically improve nor did they demonstrate change on rating scales.

Failure of baclofen to elicit an increase in plasma prolactin in these patients might be explained in several ways. Either the 80 to 120 mg/day dose (approximately 1 to 1.5 mg/kg) or one-month duration might be insufficient. In the rat, Meltzer and Fang found that only doses of 25 mg/kg and above produced increases in prolactin. Ravitz and Moore found that 5 and 10 mg/kg doses of baclofen slightly reduced prolactin, while 20 mg/kg markedly increased serum prolactin in the rat. They felt that 20 mg/kg dose was anaesthetizing in the rat and the rise in prolactin was a non-specific stress-induced effect.

It is possible that prolactin in the patients was already maximally stimulated by the presence of neuroleptics and no further change would be expected by addition of baclofen. However, five of these seven patients, while similarly stabilized on neuroleptics (chlorpromazine equivalent to 800 mg/day), showed a dramatic rise in prolactin from 51 to 117 ng/ml (53.32, $p < .05$) when treated with three gms/day of AMPT.

Although baclofen has been used successfully to treat multiple sclerosis and other neurological disorders, its mechanism of action is still open to question. This study in humans demonstrates no alteration in dopamine metabolism as reflected by plasma prolactin concentrations.

2. Animal

Imidazoleacetic Acid: Chronic administration of neuroleptic drugs to animals results in a syndrome of behavioral hypersensitivity to dopaminergic agonists. It is thought that this is a manifestation of the development of postsynaptic super sensitivity to dopamine, induced by the dopamine-receptor blocking activity of these drugs. The phenomenon of postsynaptic denervation supersensitivity has long been known to be nonspecific in regard to particular neurotransmitters; this would suggest that chronic treatment of animals with dopamine agonists would also induce alterations in behavioral sensitivity to any other neurotransmitters for which receptors are located on the same neurons as dopamine receptors. There are gamma-aminobutyric acid (GABA) receptors in the striatum, and these seem to be intimately associated with dopamine-receptive neurons. It was therefore decided to test the possibility that alterations in the behavioral sensitivity to GABA agonists accompany the behavioral hypersensitivity to dopamine that is induced by chronic treatment with the dopamine-blocking neuroleptic haloperidol. It was found that the ability of the GABA receptor agonist imidazole-4-acetic acid (IMA) to reduce motor activity was lessened in animals that had been given haloperidol chronically added to the drinking water. When the time course of the behavior of the animals were examined, it was found that the activity of the control animals was high when first placed in a novel environment, but habituated gradually over the course of testing. Animals treated chronically with

haloperidol, or injected with IMA, showed relatively small changes in total activity but essentially the same pattern of habituation. On the other hand, animals treated chronically with haloperidol and also injected with IMA showed almost a complete absence of habituation, in that their activity remained at or near its initial levels for the entire duration of a two-hour testing session. Acute injections of haloperidol had no such effect, and were simply additive with IMA in terms of decreasing behavioral activity. It is suggested that this alteration in responsivity to IMA, and presumably to the central nervous system plays a role in the therapeutic response of schizophrenic patients to haloperidol.

d. Peptides: Peptides and their relationship to the schizophrenic syndrome have been studied either by the administration of the peptides themselves, as with thyrotropin, or by the peptide's blockade, as with naloxone.

1. Thyrotropin Releasing Hormone in Chronic Schizophrenia: Intravenous administration of synthetic thyrotropin releasing hormone (TRH) has been reported to lead to a prompt amelioration of schizophrenic symptoms in a pilot study involving four female patients. Drayson gave TRH to three patients with cyclical psychoses and failed to observe any clinical change. We gave TRH to three treatment resistant chronic schizophrenic males in an inpatient research setting in which all subjects were drug free for at least a month prior to study. Synthetic TRH, 600 μg (Abbott 38579) or matching placebo, was administered by slow intravenous injection at 8 a.m. for five days followed by a two-day holiday and then crossover to the treatment not given. Order of treatment was random; neither the person giving the injection nor any of the clinical staff was aware of the nature of the substance being given. The patient's clinical status was estimated by the nurses' rating scale as well as by clinical observation by the resident psychiatrist. Particular attention was paid to each patient the first day of each series of injections because of the reported rapid onset of clinical change. Statistical comparison of active TRH vs. placebo was by t-test of the means of appropriate questions for the five days of each treatment period.

The results indicated that two of the three patients showed some worsening of their clinical condition, particularly patient 3. It is especially interesting to note that the depression score for these two patients were significantly greater during the week on TRH as compared to the week on placebo. This finding stands in contrast to earlier reports suggesting that TRH has antidepressant activity, and certainly TRH does not appear to be a useful drug for schizophrenia.

2. Naloxone: The hypothesis that endogenous opiates play a role in the schizophrenic syndrome has been tested giving naloxone, an opiate anatgonist, to schizophrenic patients. Fourteen schizophrenic patients and five patients with affective disorders (0.4 to 10 milligrams) or placebo intravenously in a double-blind fashion. Physicians' ratings of hallucinations, mannerisms and posturing, conceptual disorganization, psychosis, and mood did not change significantly. A single item, unusual thought content, improved significantly on the naloxone day compared to the placebo day. There was no improvement in mood in affectively ill patients rated either by themselves or by physicians. Naloxone did not markedly improve any patient studied, which suggests that the acute blockade of opiate receptors is not associated with global improvement in psychotic symptomatology.

e. Lithium Carbonate with Other Neuroleptic Drugs: There is good documentation in the literature indicating that chlorpromazine is superior to lithium in acute schizophrenic illness. The superiority of chlorpromazine in controlling extreme mania also appears to be estabished. Publications bearing on the use of lithium in schizo-affective illness are less unanimous in their conclusions. All of the studies so far reported do not indicate a superiority for the use of lithium over chlorpromazine in patients diagnosed as schizo-affective. There are no studies of merit using both medications simultaneously. It is our personal experience that many clinicans are using lithium carbonate in combination with major tranquilizers in the treatment of schizophrenic and schizo-affective patients, despite the fact that there are no controlled experiments in the literature bearing on the efficacy of this combination.

This study has been expanded and is taking place mainly outside the William A. White Research wards. This has been done for a number of reasons, among them our interest in being able to study a greater number of patients on low-risk protocols such as this one. After several month's work, three wards in other services have been trained in the use of the rating instruments. In addition, one of the wards is in John Howard Division. This setting offers an excellent opportunity to study the effects of lithium ion on various aspects of aggression. The studies have been completed, and we are in the process of analyzing the data and preparing the manuscript for publication.

IV. Neuroanatomy

Although the neuroanatomy of the schizophrenic syndrome is not a new area of investigation, important advances in neuroradiology (computer axial tomography) have made examinations of live subjects possible and have reawakened interest in the neuropathology of this syndrome.

A. CAT Scan

This study involves quantitative evaluations of computerized tomographic brain scans and quantitative assessments of ventricular volume and cortical sulci size determined from their scans in chronic schizophrenic patients under age 40. This provides a non-invasive, yet accurate means for considering possible abnormalities of these parameters described in prior pneumoencephalographic studies. Preliminary findings suggest that when compared to normal controls, psychiatric patients who do not meet research diagnostic criteria for chronic schizophrenia, as a group have larger ventricles and more cortical atrophy. It is also possible that in a small subgroup ventricular enlargement is dramatic.

To investigate the possibility that lateral cerebral ventricular enlargement is associated with chronic schizophrenia, computed tomography head scans from 73 chronic psychiatric patients under age 50 were compared with 56 asymptomatic normal volunteers of similar age. Ventricular size, determined by planimetry, was significantly greater in the subgroup of 58 patients diagnosed with chronic, unremitting schizophrenia than in the control group ($p < .0001$). Forty percent of the chronic schizophrenic patients were outside the control range; 53 percent exceeded two standard deviations of the control mean. Duration of illness and length of hospitalization, variables considered to be proportional to the amount of treatment received, did not correlate with ventricular size. Although patients with a history of ECT

treatment had larger ventricles than controls ($p < 0.0001$). A group of seven schizophrenic patients who were not chronic also had enlarged ventricles ($p < .006$); eight patients who were either schizo-affective or not schizophrenic did not differ from controls.

This study demonstrates that lateral cerebral ventricular enlargement is associated with chronic schizophrenia; it suggests that this finding is not a result of treatment. Computed tomography was further employed to investigate whether mild cerebral atrophy involves the cerebral cortex. The widths of the sylvian fissure, the interhemisphere fissure, and three cortical sulci were measured blindly on computed tomography (CT) scans from 75 chronic psychiatric patients and 62 asymptomatic volunteers all under age 50. Using dimensions from the control subjects to define the range of normal size for these structures, 19 (32%) of the 60 patients diagnosed with chronic schizophrenia had at least one abnormality. All 15 patients with other diagnoses were within the control range. Comparing those chronic schizophrenic patients with abnormalities to those without them, there were no significant differences in age, length of illness or treatment, and length of hospitalization. Combining the previously reported data on ventricular size with the present findings, two-thirds of the chronic schizophrenic patients had some abnormality of cerebral structure evident on CT scanning. Ventricular enlargement, however, did not correlate significantly with cortical abnormalities. It is suggested, therefore, that more than one etiology may account for the structural abnormalities found in chronic schizophrenic patients. Furthermore, we also found, totally unexpectedly, a subgroup of 10 chronic schizophrenic patients with possible pathology of the cerebellum.

The scans of five chronic schizophrenic patients were interpreted by a neuroradiologist as showing definite atrophy of the vermis, in one of these patients, the cerebellar hemispheres were also considered atrophied. Five additional scans, which were interpreted as "questionable", met objective criteria proposed for the diagnosis of vermian atrophy on a CT scan. One of these patients had a history of alcohol use. Since cerebellar atrophy is common in chronic alcoholics this patient was excluded from further study. None of the remaining nine patients (four females, five males; mean age 35, range 26-44) had an identifiable cause for these findings; no patient was taking medication other than neuroleptics. Furthermore, careful neurological examination with emphasis on cerebellar function revealed abnormalities in only three patients, two with mild dysdiadokokinesis of the non-dominant hand, and one with a slight positional tremor of both hands.

A growing body of basic science research indicates that the cerebellar vermis is linked anatomically and functionally to areas of the limbic system that have been implicated in schizophrenia. Furthermore, increased dopamine in the limbic forebrain, a neurochemical abnormality consistent wtih the "dopamine hypothesis" of schizophrenia, has been produced in the rat by a lesion of the vermis. It has also been reported that in several schizophrenic patients, electrical stimulation of the vermis had therapeutic effects. Such observations suggest that pathology of the cerebellar vermis may be related to the pathogenesis of some schizophrenic symptomatology.

In a morphometric study of the anterior cerebellar vermis of 46 brains in the Yakovlev Collection, the area of the vermis of five of 12 brains of schizophrenic patients was smaller than any of 11 controls without psychiatric or neurologic disease

and eight of nine control with other psychiatric diagnoses ($p < .02$). This finding confirms computerized tomography scan observations in live patients and indicates that some schizophrenics have structural abnormalities of the cerebellar vermis.

Four of the 17 chronic schizophrenic or chronic schizoaffective patients between the ages of 20 and 35, in partial remission, and living in the community were found to have mildly or moderately prominent sulci, as disclosed by computerized tomography (CT scan). These four patients were matched for sex and age with four chronic schizophrenic or schizoaffective patients without sulcal prominence. The Halstead-Reitan battery (HRB) of neuropsychological tests, including the Wechsler Adult Intelligence Scale (WAIS), was administered to these eight patients with the examiner blind to results of the CT scan. Each of the four patients with sulcal prominence considerably exceeded an established cutoff score for brain impairment on the HRB, whereas only one of the control schizophrenics performed in the impaired range, and that just beyond the cutoff. The WAIS Verbal IQ, measured concurrently, was in the normal range for all subjects and similar for the two groups. These findings suggest that mild or moderate sulcal prominence on CT scans among young chronic schizophrenics is associated with impaired neuropsychological functioning.

B. HLA Antigens

A survey of 14 published studies found no consistent association between HLA antigens and schizophrenia. Since the studies lacked both a shared diagnostic system or biological criteria, an investigation was undertaken using Research Diagnostic Criteria and CT scan findings. Typing for HLA antigens at loci A, B and C was carried out on 126 patients. Among 64 black schizophrenic patients there was an increase of HLA-A_2 that remained significant even after correcting for the number of antigens studied. When the patients for whom CT scans were available was divided according to the presence or absence of evidence of brain atrophy there was an increase of A_2 with black schizophrenic patients without atrophy (70% vs 30%) that remained significant after multiplying by the number of antigens studied but there was no significant increase of A_2 on those with atrophy. Similar trends held for the white population but they failed to reach significance. The need for HLA studies on biologically defined groups of schizophrenics is stressed.

Several diseases have been shown to be associated with specific human leukocyte antigens. While there have been a number of reported associations between the HLA system and schizophrenia, none of these has been consistent. This lack of consistency could be for a number of reasons including: 1) differences in diagnostic systems and 2) biological differences among schizophrenics.

C. Laterality

On the basis of a study of handedness in monozygotic (MZ) twins at least one of whom was schizophrenic we hypothesized the existence of a subgroup of schizophrenic patients characterized by anomalous lateralization and a milder form of illness. To test this hypothesis we examined laterality in a group of schizophrenic patients.

Our sample consisted of 66 inpatients at Saint Elizabeths Hospital, Washington, D.C. who met Research Diagnostic Criteria (RDC) for schizophrenia or schizo-

affective psychosis. The schizophrenic patients were further subdiagnosed into chronic (present episode of at least one year duration or incomplete recovery from previous episodes) and acute (present episode less than one year or complete recovery from previous episodes) by a rater unaware of the patients laterality. The patients were tested for laterality on a 12 item test. Those who used their right eye, hand or foot on nine or more items were considered right handed, the others were considered not right handed (left handed or ambidextrous). Also a subgroup of 55 were given the Torque Test which consists of drawing three circles with each hand. Those who drew a circle in the clockwise direction with either hand were said to have Torque. Showing Torque is thought to be a left-handed characteristic and in behaviorally disturbed children has been shown to be associaed with a figure diagnosis of schizophrenia.

The results indicate that anomalous lateralization, as determined either by a battery of tasks or by the Torque test, appears to be associated with a milder form of schizophrenia. This is similar to the findings of Lishman and McMeekan who found an increase in left handedness in schizo-affective psychoses but not "true" schizophrenia. It is also in keeping with our hypothesis that there exists a subgroup of schizophrenic patients with a milder form of illness who show anomalous lateralization. As a corollary, these results suggest that in severely ill schizophrenics laterality disturbances may not be more prevalent than in the general population.

If more interpretation is correct it has several implications. First, it suggests that the proportion of left handedness in a group of schizophrenics will vary depending on the percentage of milder forms of schizophrenias included in this sample. It is also possible that anomalous lateralization is important in the etiology of some schizophrenias. A study of MZ twins with schizophrenia shows that in twinships with at least one left handed twin the left handers tend to suffer from a mild schizophrenia and the right handers are not schizophrenic. This suggests that left handedness may, in some individuals predispose to schizophrenia. Also reversed neuroanatomical asymmetries on computed tomography scans, (another measure of anomalous lateralization) have been found with increased frequency only in those schizophrenic patients who show no evidence of brain atrophy. This supports the notion of etiological heterogeneity of schizophrenia and the possibility that anomalous lateralization is etiologically significant in some schizophrenic patients.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01337-10 SMRA

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies on Drugs of Abuse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project continues to be investigation of the mechanism of actions of drugs of abuse. Our hope is that increased understanding of drug effects can help us further differentiate normal mental function and mental illness.

The report that follows contains brief summaries of research initiated and the update on continuing work in our laboratory during 1980. The summaries have been broken down into the following catagories:

I. Alcohol Abuse
II. Hemsipheric Differences
III. Hypnotics
IV. Opiate Antagonists and Agonists
V. Central Nervous System Stimulants and Hallucinogens

These catagories are divided, again, under the subheadings of the specific studies.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The objective of research performed by the Laboratory of Clinical Psychopharmacology is to further our understanding of the nature and action of pharmacologic agents classified as drugs of abuse. Through research investigating the mechanisms of these substances, we hope to contribute to the body of knowledge concerning their usage and effects.

I. Alcohol Abuse:

A. Benzodiazepines

The benzodiazepines are the most commonly prescribed drugs in this country, particularly for the elderly. Routine monitoring of benzodiazepine concentrations is desirable for may reasons. Patient dose requirements vary widely, which may be due to the large range of blood levels reported following the same dose. Because of benzodiazepine side effects, lethality when mixed with alcohol, and the risk of physiological and psychological dependence appearing dose related, patients should be maintained on the lowest effective therapeutic dose. The principal method for measuring benzodiazepines, gas liquid chromatography, has not attained routine clinical use because of its technical complexities. Also, development of a specific assay is required for each drug and its metabolites. We describe here an assay for benzodiazepines in saliva based on competition for diazepam receptor binding.

In this assay, saliva is used without any extraction or precipitation. Saliva is first contrifuged to separate it into mucous sediment and serous supernatant. The serous supernatant is diluted and added directly to the assay. For the benzodiazepine radioreceptor assay, fresh rat frontal cortex was homogenized. The homogenate was centrifuged twice with rehomogenization of the intermediate pellet in fresh buffer. The final pellet was resuspended and diluted in fresh buffer. Polypropylene incubation tubes received diluted saliva or the drug solvent buffer, and tissue suspension. Final concentration of ^{3}H-diazepam was 0.50 nM.

Benzodiazepines in saliva reduce the specific binding of ^{3}H-diazepam more than the small degree of inhibition caused by drug-free saliva, and the amount of inhibition of specific binding is proportional to the amount of benzodiazepine in saliva. The amount of benzodiazepine in a saliva sample can easily be determined using a standard curve of inhibition of ^{3}H-ligand binding by known amounts of the drug.

This method requires that benzodiazepines present in saliva will not inhibit non-specific binding of ^{3}H-ligand. Thus, specific binding in the presence of patient saliva can be determined by subtracting non-specific binding values obtained with control saliva from total binding with patient saliva. The percentage inhibition of specific ^{3}H-diazepam binding (in the presence of control saliva) by the patient saliva (containing drug) is then calculated and compared with a standard displacement curve for determining actual benzodiazepine content.

Similar reductions in specific and non-specific binding of ^{3}H-diazepam by saliva indicates that saliva components may bind benzodiazepines making less available for membrane binding. In this assay, benzodiazepines bound to saliva components dissociate during the incubation so that total benzodiazepine levels are measured.

The validity of the radioreceptor assay was assessed by comparing its results with saliva levels measured by another procedure. We measured saliva benzodiazepine levels in patients treated with oxazepam; saliva oxazepam levels were measured on the same samples by gas liquid chromatography with electron capture and by saliva radioreceptor assay. We found a good agreement between the different methods.

In summary, the simplicity of the benzodiazepine receptor assay for saliva, as well as the ease with which saliva samples can be collected and stored, make this assay suitable for routine clinical use in large patient populations. Saliva can be collected at home and refrigerated for later assay.

An advantage of this assay is that it will detect benzodiazepine metabolites that compete for ^{3}H-diazepam binding and which may thus be clinically active. There is evidence that a major part of diazepam's clinical efficacy is due to its metabolites desmethyldiazepam and oxazepam. Both bind to the diazepam receptor and will thus be detected in this assay.

The radioreceptor assay for benzodiazepine in saliva is simpler to perform than would be a radioreceptor assay for benzodiazepine in plasma or red blood cells. We have found a high degree of inhibition of ^{3}H-diazepam specific and non-specific binding by both plasma and red blood cell lysates.

Employing this benzodiazepine radioreceptor assay, a group of volunteer adult alcoholic patients undergoing inpatient detoxification were evaluated at initiation and termination of benzodiazepine treatment (time 1) by the Hamilton Anxiety Scale and the Hopkins Symptom Checklist. An initial saliva collection was made the morning following the first benzodiazepine administration. On the day prior to termination of benzodiazepine treatment or discharge from the hospital (time 2), a re-evaluation of clinical state was made by the treating physician with the Hamilton Anxiety Scale and Clinical Global Impression Scale. The patients also completed a Hopkins Symptom Checklist at this time. On the morning of this clinical evaluation, a second saliva sample was collected and benzodiazepine saliva concentration determined.

The pretinent data from this study are summarized in Table 1 and Figure 1. The subjects were divided into two groups based on outcome assessed by the Hamilton Anxiety Scale and the Hopkins Symptoms Checklist. Those patients who had either less than ten percent decrements or increments in scores from time 1 to time 2 were empirically classsified as benzodiazepine nonresponders (n=6). All other subjects were classified as benzodiazepine responders (n=12).

As can be seen in Figure 1, the responder group has significant differences in saliva benzodiazepine concentrations compared with the nonresponder group measured at time 2. Although the responder group had relatively greater mean saliva benzodiazepine concentrations that the non-responder group at time 1, this difference failed to achieve statistical significance. Furthermore, as is seen in Table 1, the

responder group had significant correlations between saliva benzodiazepine concentration and benzodiazepine dosage. There are no differences in mean benzodiazepine dosage between the two groups. Additionally the correlation between assessment scales (Hamilton and Hopkins) in the nonresponder group indicates that those patients who achieve the lowest benzodiazepine saliva concentration have the highest psychopathology scores. In contrast, among the responder group, there is a direct relationship between saliva benzodiazepine concentration and psychopathology scores at time 2. As can be seen in Table 1, the first order partial correlation analysis, controlling for clinical ratings at time 1, indicates that the initial psychopathology scores obscure the eventual relationship of saliva benzodiazepine concentration to psychopathology scores in both groups. Since the first evaluation is made easily in the course of treatment, these ratings may be more reflective of a potentially wide variety of psychoactive agents, including alcohol, present in this group at the time of admission. It is difficult to speculate (e.g., upper therapeutic window) in a preliminary study such as this about the meaning of a direct relationship between saliva concentration and psychopathology scores but it is a phenomenon that warrants further study in a larger, more rigorous investigation.

Table 1

CORRELATION ANALYSIS OF SALIVA BENZODIAZEPINE ASSAY

Responders (n=12)

	Hopkins[2]	Hamilton[2]	Global[2]	Dose[2]	Cum. Dose[2]
Saliva[2]	.41 NS	.40 NS	.35 NS	.70 p=.01	.53 p=.07
1st Order Partial Corr (initial psychopath-pathology scores)	.57 p=.05	.53 p=.07	.43 NS	.71 p=.01	.51 p=.08

	Nonresponders (n=6)				
	Hopkins^2	Hamilton^2	Global^2	Dose^2	Cum. Dose^2
Saliva^2	-.16 NS	-.22 NS	.14 NS	-.42 NS	-.39 NS
1st Order Partial Corr (initial psycho-pathology scores)	-.53 p=.08	-.72 p=.02	-.38 NS	-.26 NS	-.27 NS

KEY:
Saliva^2=Saliva benzodiazepine, time 2
Hopkins^2=Hopkins Symptoms Checklist, time 2
Hamilton^2=Hamilton Anxiety Scale, time 2
Global^2=Clinical Global Impression, time 2
Dose^2=Benzodiazepine dosage, time 2
Cum Dose^2=Cumulative benzodiazepine dosage, time 2
NS=Nonsignificant

B. Radiographic and Neuropsychological Correlates of Alcohol Use

Despite the growing awareness about the social and medical hazards associated with alcohol abuse, there remains much to be learned about its effective treatment and prevention, as well as about the biochemical and psychosocial effects. One area of research in which information is needed is on interactive effects of brain structure, peripheral measures of catecholamine function and alcohol upon intellectual functioning. By using recent developments in neuropsychology and neurochemistry, we hope to provide data that will enhance our understanding of the relationships among alcohol consumption, catecholamine function and intellectual processes.

Recent reviews of neuropsychological deficits in alcoholics have concluded that overall intelligence is rarely impaired. Significant deficits appear, however, in specific nonverbal behaviors such as performance of complex perceptual-motor tasks. Also, to date, research on the neurotoxic concomitants of alcohol use in humans has not systematically examined the precise relationship between alcohol consumption and central nervous system deficits. Recent studies have reported, though, that alcohol consumption well below that of alcoholic patients is significantly associated with impaired neuropsychological performance. In fact, these data indicate that even amounts of alcohol consumed by social drinkers may increase age related deficits in intellectual processes. Therefore, by assessing levels of alcohol consumption associated with structural, chemical and behavioral indices of central nervous system abnormalities, we hope to increase our understanding of alcoholic brain damage as well as cognitive deficits associated with subalcoholismic levels of consumption.

Subjects will be 200 males or females, over 21 years of age, (we will over sample the elderly) who have received brain CT (computerized tomography) scans at Suburban Hospital for medical reasons. No patient who has suffered recent brain injuries or who has disorders that will interfere with their taking the brief neuropsychological battery (e.g., severe sensory or motor impairements) will be included. Subjects are now being selected.

For each subject, the following information will be obtained:

(1) alcohol use
(2) neuropsychological performance
(3) neurochemical assessment from blood samples
(4) education level
(5) age
(6) current medications
(7) diagnosis
(8) brain scan

It is anticipated that the distribution of drinking practices of patient-volunteers will not deviate significantly from the normal population. Thus, we project that approximately 30% will be abstainers, 60% light to moderate drinkers, and 10% heavy drinkers. Subjects from all categories of drinkers will be included in this study, since the purpose is to examine the continuum for drinking practices in relation to CT scans and neuropsychological performance.

Accumulating evidence points to substantial associated changes of a cognitive and structural nature with advancing age and/or abusive alcohol use. Little information is extant concerning these changes in the vast majority of alcohol users. We hope that data from this study will provide valuable information to the medical community to advise and/or treat their patients with respect to alcohol use.

II. Hemispheric Differences

From research discoveries we have made previously comparing marijuana and alcohol effects on hemispheric function, we have expanded our study of hemispheric specialization. Functional specialization of the cerebral hemispheres in man is widely assumed to be fixed by adulthood. The emergent lateralization, however, of "same"/"different" judgements in adult subjects suggests that not every instance of hemispheric specialization is an outcome of childhood development. Rather, the adult human brain retains the plasticity required for additional hemispheric differentiation. This differentiation may not be fully achieved until a subject has performed a considerable number of trials, far more than are customarily used to measure "laterality".

Our subjects were 16 males (aged 19 to 29) who were right-handed as assessed by the modified Edinborough Handedness Inventory. They sat in front of a projection screen and were requested to fixate on a red warning light that appeared briefly (500 msec) in the center of the screen immediately before each tachistoscopic projection of the test letters. They appeared for 100 msec, and the subjects were asked to indicate as quickly as possible whether the two letters were the "same" or "different". They did this by depressing a telegraph key with the right index finger for "different" and

with the right thumb for "same". Reaction time was measured as the interval between the opening of the shutter to the closing of the telegraph key. Three seconds separated each slide.

Subjects were tested during four sessions a day for three days, with a week between each testing day. The first two sessions were separated by 10 minutes, sessions two and three by 30 minutes, and sessions three and four by four hours. The purpose of this schedule was to replicate as closely as possible the conditions of the earlier drug experiment in which the hemispheric differentiation reported here first became evident.

Our most striking finding was the development of opposite hemifield advantages for "same" and "different" judgments. Over the three days of 4,608 trials, "same" judgments were made with increasingly distinct left visual field advantage; "different" judgements with increasingly right visual field advantage. (In man, stimuli seen in the right or left visual field are relayed exclusively to the visual cortex of the opposite hemisphere. Although the paths of subsequent transmission in the brain are far from clear, we know, for example, that left field stimuli are right hemisphere entrant). Differentiation proceeded equally rapidly for both responses: Subjects responded to "different" stimuli in the right visual field with a reaction time advantage which closely mirrored the advantage for responses to "same" stimuli in the left visual field.

These findings closely replicate those found in our previous drug study. A different group of 16 subjects, who were moderate users of both alcohol and marijuana, were tested using the same procedures, but were given either a marijuana cigarette containing 15 mg of tetrahydrocannabinol or a drink containing 1 gram of ethanol per kilogram of body weight between the second and third session of each day. The data from this previous study also shows gradually developing hemispheric asymmetries of "same"/"different" judgments which are remarkably similar to the non-drug group in both magnitude and direction. The congruence of these groups in days two and three shows that neither a history of drug use nor actual intoxication during the trials affects the overall progression of lateralization of the two responses. (Whether this is a characteristic differentiating drug users from non-users in unknown).

Correct interpretation of these findings is extremely important, but cannot be completed using the data at hand. Subsequent studies will examine the phenomenon of increasing cerebral asymmetry over repeated trials and whether it is based on specialized hemispheric abilities (for "same" versus "different"), on differentiated memory stores, or on motor allocation. What is clear from the present data is that asymmetric cerebral specialization can develop in adults during repeated trials.

Another direction our work has taken investigating hemispheric specialization involved the principle of Torque. Individuals who draw circles in a clockwise direction are described as showing Torque. The frequency of Torque positive individuals is increased in children who are left-handed, who have behavioral or learning disorders and who on follow-up are diagnosed schizophrenic.

We have shown in previous research, also, that adult schizophrenic patients with Torque are similar to schizophrenic patients who are not fully right handed in that both groups have a milder and less chronic form of illness. Thus, the presence of

Torque appears related to left handedness, a variety of psychological disturbances, and possibly some forms of schizophrenia. These findings provide further evidence linking abnormalities in lateralization with psychiatric illness and specifically schizophrenia. Since the nature of this link remains unclear, however, we felt that it would be warranted to examine how the presence of Torque is related to hemispheric function. This was made possible by designing a study which included a test of Torque and a test of hemispheric function.

The subjects were 15 male volunteers (aged 19 to 29) who were shown to be right handed on the Edinborough Handedness Inventory. Each was presented with a sheet of paper with two rows of three small x's and told to draw circles around one row with the right hand and the other row with the left hand. Eight of the 15 drew at least one circle in the clockwise direction, and were thereby considered to have Torque. The other seven (7) drew all the circles in the counterclockwise direction and were considered not to have Torque. The subjects then participated in a test of hemispheric function. It involved the subjects fixating straight ahead while slides were presented tachistoscopically to either of their visual fields. They were instructed to press, as rapidly as possible, either of two telegraph keys depending on whether the slide displayed letters that were similar or different. Each subject was tested for four sessions each day for three days, with a week between each test day.

As the results show in Table 1, the mean reaction time averaged over all trials differed for the Torque positive and Torque negative groups. The Torque positive group tended to have shorter reaction times on day 2 ($p=.06$) and significantly shorter reaction times on day 3 ($p \sim .02$).

The second significant effect of Torque was its interaction in a 2 x 2 ANOVA with the hemisphere to which information was flashed. Table 2 shows that the Torque positive group had an asymmetry favoring information presented to the right visual field and therefore being processed by the left hemisphere, compared to the Torque negative group which showed an asymmetry favoring information presented to the left visual field.

In the original study, it was found that hemispheric asymmetry appeared to develop over the course of the three test days and was the most robust on the last day. The present report shows that the same trends hold when the data are grouped by subject on the basis of Torque.

The first effect noted was that reaction time was shorter for the Torque positive group. This effect is not related to an increased error rate. There was no significant difference in the number of errors between the Torque positive and negative groups on any of the three days.

The second finding indicates that there is a difference in hemispheric functioning between the Torque positive and negative groups. It suggests that the Torque test measures differences in cerebral lateralization. Also, since all the individuals tested had been found to be right handed on the Edinborough Handedness Inventory, the results suggest that the Torque test is able to tap some feature of cerebral lateralization that is not readily detected by standard measurements of handedness. We have commented in the past on the variability noted in studies of cerebral

lateralization. In particular, we stressed the need to test over an extended number of trials. An additional factor that may need to be controlled is the distribution of Torque in the population tested.

Our results suggest that the Torque positive group tends to show an asymmetry favoring information presented to the left hemisphere. Thus this group does not show evidence of left hemispheric deficit. Yet, as noted in the introduction, Torque is more frequent in a variety of conditions (left handedness, behavioral and learning disorders in children and schizophrenia) which have been hypothesized to involve a left hemispheric deficit. Our results go against a link between Torque and left hemispheric deficit. If anything, our results suggest that those with Torque have left hemispheric "bias" and "overactivity". It is interesting that studies of lateralized cerebral function in schizophrenic patients using a variety of paradigms have been interpreted to reflect left hemispheric "overactivity". Whether schizophrenia is associated with left hemispheric deficit and/or "overactivity", it would seem appropriate in future studies of hemispheric function in schizophrenia to control for Torque test scores in order to insure that differences noted between schizophrenics and other groups are related to their illness and not to the presence or absence of Torque. Our results also suggest that hemispheric asymmetry with apparent left hemispheric advantage may not be specific to schizophrenia, but may occur in normal right handers who show Torque. These hemispheric asymmetries might also be studied in other populations with an increased incidence of Torque including left handers and children with behavioral and learning disturbances.

Table 1

TOTAL MEAN ($\pm$ S.D.) REACTION TIME (MILLISECONDS)

IN TORQUE POSITIVE AND NEGATIVE SUBJECTS

	Day 1	Day 2	Day 3
Torque + (n=8)	776 $\pm$ 160	660 $\pm$ 126	619 $\pm$ 104
Torque - (n=7)	840 $\pm$ 147	763 $\pm$ 117	727 $\pm$ 105
	F=.092, Df=1, p=.35	F=4.0, Df=1, p=.06	F=7.08, Df=1, p$\sim$.02

Table 2

MEAN (± S.D.) REACTION TIMES (MILLISECONDS) FOR SLIDES

PRESEENTED TO EACH HEMISPHERE IN TORQUE POSITIVE AND NEGATIVE SUBJECTS

		Day 1	Day 2	Day 3
Torque + (n=8)	Left Hemisphere	778 ± 162	656 ± 123	613 ± 103
	Right Hemisphere	767 ± 159	664 ± 128	624 ± 105
	Difference Right Hemisphere minus Left Hemisphere	+11	+8	+11
Torque - (n=7)	Left Hemisphere	840 ± 144	770 ± 113	732 ± 102
	Right Hemiphere	840 ± 150	757 ± 120	723 ± 108
	Difference Right Hemisphere minus Left Hemisphere	0	-13	-9
		F=.04, Df=1, p=.836	F=5.84, Df=1, p< .03	F=10.77, Df=1, p <.01

III. Hypnotics

In April 1979, the Institute of Medicine, National Academy of Sciences issued its report, Sleeping Pills, Medical Practice, and Insomnia, the result of a study lasting over one year. Dr. J. Christian Gillin was a member of the Steering Committee, and Dr. Wallace Mendelson was the chief technical consultant. Dr. Wyatt advised the committee, also. This study was inspired by NIDA's previously published report. It drew attention to some of the less publicized unanswered questions and hazards associated with long-term use of benzodiazepines, such as interaction with alcohol, accumulation of long-term metabolites, adverse effects in the elderly, and the indications for hypnotic use. As a result of his participation in this study, Dr. Mendelson has written a book, The Use and Misuse of Sleeping Pills, published 1980, by Plenum Press. The laboratory has had a long interest in developing less dangerous, more efficacious pharmacological treatments for insomnia. We were among the first to suggest that L-tryptophan functions as a "natural hypnotic". We have also studied the effect of the four major "hypnotoxins" in the rat: Pappenheimer's Substance S, Veonnier's DSIP, Peve's arginine vasotoxin, and Nagasacki's extract from sleep deprived brain. We have not been able to demonstrate an hypnotic effect for any of these substances.

In December, 1979, the Surgeon General initiated Project Sleep, a three-year joint public-private effort to upgrade the quality of clinical care provided to patients with clinical sleep disorders. It will be primarily an educational program aimed at the

health professions. Drs. Gillin and Mendelson are members of the Steering Committee which is chaired by Dr. Steven Kennedy.

Among the activities of Project Sleep is a National Consensus Conference addressing a broad range of issues related to sleep problems and depression in the elderly. Drs. Mendelson and Gillin are developing, also, teaching materials to be used in medical schools in the area of sleep disorders.

IV. Opiate Antagonists and Agonists

A. Basic Studies

Various molecular forms of enkephalin-like peptides have recently been detected in sympathetic ganglia of rats, guinea pigs and rabbits and in the adrenal medulla of several species. Since the rate of catecholamine metabolism in peripheral catecholamine neurons relates to the activity of these neurons, we wondered whether the activation of opiate receptors by endogenous ligands such as the enkephalin-like peptides produced in adrenal medulla could change the activity of sympathetic neurons and thereby influence the metabolic rates and peripheral catecholamines. We attempted to answer this question using the effects of morphine on sympathetic ganglia as a model. Sympathetic ganglia contain adrenergic and dopaminergic neurons and, therefore, they are excellent tissues inwhich to study the consequence of opiate receptor activation on peripheral catecholamine metabolism. As an opiate receptor agonist, we used morphine because it is more stable than the enkephalins. To evaluate whether blood borne enkephalin-like peptides secreted from adrenal medulla regulate peripheral catecholamine stores we compared the turnover rate of catecholamines in normal and decentralized superior cervical ganglia of demedullated and intact rats.

The turnover and metabolism of catecholamines after intraperitoneal administration of 10 (low dose) or 40 (high dose) mg per kg morphine sulfate were measured in the brain, lumbar spinal cord, heart, celiac, and superior cervical ganglia of demedullated rats with unilaterally decentralized superior cervical ganglia. Demedullation significantly increased norepinephrine concentration and turnover in both sham operated and decentralized superior cervical ganglia, but demedullation failed to change the turnover of dopamine in both ganglia. Low doses of morphine only increased dopamine metabolism in the caudate nucleus. High doses of morphine failed to change the turnover of norepinephrine stored in heart and sympathetic ganglia but produced variable changes in norepinephrine turnover in the spinal cord and brain. High doses of morphine significantly increased dopamine turnover and metabolism in the celiac, superior cervical ganglia, caudate nucleus, and the spinal cord of demedullated rats. These effects of morphine were antagonized by naloxone. Dopamine metabolism in decentralized superior cervical ganglia of demedullated and intact rats remained unchanged after high doses of morphine. The action of morphine on dopamine metabolism was facilitated by demedullation, suggesting that the adrenal medulla secretes a modulator that can counteract the effects of opiate ligands on ganglionic small intensity fluorescent cells. Atropine and hexamethonium failed to antagonize the increased in the metabolism of ganglionic dopamine produced by morphine; and the increased in ganglionic dopamine metabolism caused by oxotremorine was not antagonized by naloxone, thus confirming a disassociation between the action on ganglionic dopamine mediated by cholinergic and opiate

receptors. Since decentralization of the superior cervical ganglia abolished the increase in dopamine metabolism induced by morphine, stimulation of extraganglionic opiate receptors reflects the metabolism of dopamine located in small intensity fluorescent cells.

B. Clinical Studies

Naloxone has been reported to reduce auditory hallucinations and thought disorder in chronic schizophrenic patients. We have studied this by examining changes in psychiatric symptomology of schizophrenics after naloxone versus saline infusion. In an initial study, 0.4 mg of naloxone was infused in five patients. We have now increased the dosage to 15 mg with i.v. infusion with the thought that such an increase in dosage would decrease symptomology, especially hallucinations. In fact, the increased dosage has produced a subclinical decrease in the abnormal thought content item on the Brief Psychiatric Rating Scale (BPRS).

To date, we have increased our number of patients to 27, all patients having been diagnosed as chronic schizophrenic using Research Diagnostic Criteria (RDC). In the drug-free state (as opposed to drug-free affective patients or control subjects), the schizophrenic group showed plasma growth hormone and prolactin elevations from 15 through 60 minutes. When this patient group was studied while receiving antipsychotic medication, no prolactin increased elevation was observed, but, a delayed growth hormone rise (45 through 120 minutes) occurred. Of note, a subclinical abnormal thought content decrease was seen in the currently medicated patient group. Moreover, a few medicated patients demonstrated a significant clinical diminution in auditory hallucinations. One patient in particular could identify the exact time the last hallucination occurred (two hours after infusion which lasted up to six hours).

In a subgroup of schizophrenic patients can be identified, whose symptoms are ameliorated by naloxone, a more chronic administration would be used. This approach of screening for specific response to naloxone would be extrapolated to oral naltrexone and beta-endorphine. Other than the one significant responders, no other patient has shown such a beneficial response. Our screening of new drug free patients continues.

IV. Central Nervous System Stimulants and Hallucinogens

Dr. David Stoff, principal investigator in animal studies of abused drugs, in concert with other colleagues, has evaluated the animal models that have been used for drug-induced hallucinations. Criteria have been proposed which should be met by an "ideal" animal model. The data suggest that some of the behavioral disturbances in animals caused by amphetamines and hallucinogens can be explained by a deficit in attention and/or information processing. We have discussed, also, the interaction of hallucinogens with other drugs, such as the transmethylation hypothesis of schizophrenia, the role of indole hallucinogens in schizophrenia and the relevance of phenylethylamine to drugs of abuse and neuropsychiatric disorders.

In this country, there has been a steady increase in the nonmedical popularity of central nervous stimulants (amphetamine-related compounds, cocaine) and hallucinogens (phencyclidine, LSD) over the last decade. A parallel increase in laboratory research aimed at elucidating the mechanism of action of these drugs and developing

effective treatments is evidenced by the wealth of data in the literature which describe the physiological, pharmacological, behavioral and biochemical actions of psychomotor stimulants and hallucinogens. Most of this work, however, has been considered primarily by characterizing the acute effects of these drugs, although it is the chronic state of intoxication that, clinically speaking, leads to psychosis and movement disorders. Chronic amphetamine intoxication is an insidious problem in the majority of cases. Symptoms usually take a long time to appear and daily intake of drugs by chronic abusers can become quite high before significant problems appear. Eventually, however, the drug abuser suffers personality changes, weight losses and marked impairment of cardiovascular, central nervous and gastrointestinal systems.

Current information suggets that chronic use of some central nervous system stimulants and hallucinogenic agents lead to more exaggerated catecholaminergic effects than single administration (instead of tolerance as is the case with the central nervous system barbituarates, alcohol, benzodiazapines and the narcotic analgesics). This is based on animal experiments after chronic drug exposure using electrophysiological, behavioral and neurochemical measurements. These data bear out the growing concern about the psychological (not physical) dependence engendered by the chronic use of these drugs and possibility of the structural damage caused by continuous drug exposure.

It is well known that psychoactive drugs have a variety of psychological effects, both "wanted and unwanted". These effects have been referred to as "euphoria", "good trip", "psychedelic", "edietics", versus "dysphoria", "bad trip", "psychotomimetic", "psychotoxic". These different effects may work through different mechanisms, involve different neural substrates, occur under different enviornmental conditions, and require different treatment modalities. Because the pleasant effects (i.e., "relaxed" alertness, increased energy, euphoria for stimulants; perceptual changes, transcendental state for hallucinogens) usually occur at relatively low doses after acute administration, there is a tendency (which can become a "craving") to use higher dose and/or more frequent drug administrations in order to intensify the effects. The euphoria, however, is rapidly replaced by psychological depression, motivating users to repeat the dose very frequently in an attempt to maintain a continuous "high". The individual is then caught in the bind of continually taking the drugs to relieve, presumably, the state of tension or dysphoria, when the original motivation was to continue drug-taking to produce a better euphoria. Because chronic stimulant use is often accompained by the development of "reverse tolerance", with increasing sensitivity to the drug's effect, this in turn may induce excitatory symptoms (at certain doses) and prompt the development of agitation, anxiety, paranoid thoughts, auditory and visual hallucinations and, at times, violent, irrational behavior. Furthermore, repeated drug use may change the response to subsequent drug administration. With long-term use, tolerance does not develop to the dysphoric and/or psychosis-inducing effects and chronic users either experience less euphoria or become more sensitive to the dysphoric and/or psychotomimetic effects. Sensitization causes a shift on the dose response continuum so that a previous euphoriant dose becomes dysphoriant.

Due to our interest in the chronic state of intoxication in humans, we decided to construct a "model" behavioral system for small animals which:

(1) Continuously monitored every aspect of the laboratory animal's behavior (without any intrusive techniques) in order to get the animal's life history. We focused on behavioral parameters that bear some relevance to the chronic stimulant and hallucinogenic drug states and show some change in response to chronic treatment;

(2) Provided both a home and experimental situation to mimic the laboratory animal's habitat as much as possible;

(3) Easily allowed for continuous drug administration without any intervention and the stimultaneous monitoring of ongoing behavioral changes; and

(4) Had been reasonably well characterized as an experimental situation making it possible to ask some relevant conceptual questions.

Methods:

1. Activity System for Longitudinal Measurement

We have developed an activity system which is a more precise, objective and practical technique than traditional observational methods in measuring drug-induced motor changes. The level of behavioral complexity which can be monitored is limited primarily by our ability to define the phenomena.

(a) Description

The activity system is schematically diagrammed in Figure 1 and 2. The animals live in a square plexiglass cage with access to food and water. This cage is housed in a shielded enviornmental enclosure which provides ventilation and lighting. Conductive surfaces which are mounted outside and parallel to the plane of each plexiglass wall operate with the grounded stainless steel rod floor to generate four functionally electric fields inside the cage. The animal's location in the cage is transduced by the four physically overlapping but functionally separate electric fields within the cage. The animal distorts each field according to his proximity to each plate thus affecting the value of each capacitance. Electronic circuitry generates from these values two analog voltages which represent the animal's location along x-y axis of the cage floor (Figure 3). A microprocessor transforms these signals into linear values which are processed by programs (algorithms for rear response, gross movements, and fine movements), reflecting the position of the animal and occurrence of the specific response. The program is burnt on a permanent read-only memory in this mini-computer to recognize and record activity information. This information is displayed, in digital form, on a terminal and is also stored on a tape cassette. The information on cassette can then be transferred to a PDP 11-34 which prints out a hard copy of the data and does the appropriate statistical analyses.

(b) Calibration

D-amphetamine was injected (ip) in naive Fischer 344/Mai rats (male, 200 g) and thresholds were empirically determined by calibrating different threshold settings with behavioral observations (Table 1). Two movement thresholds were used to differentiate between fine and gross body movements. The fine movement yielded high numbers which remained stable during peak while continuous, restricted amphetamine-induced stereotypes were rated. There was an inverse relationship with increases in threshold settings (from 2.5 to 40 mm) correlated with decreases in computer counts (from 328 to 0) (Table 1). The actual vector lengths associated with those stereotypies (from 2.5 to 20 mm) can be detected over a very narrow range.

Table 1

Calibration Of Fine Or Gross Movements And Rear Response Legend

Threshold Value	Computer Counts	Threshold Value (mv)	Computer Counts
2.5	328 ± 19	2000	140
5	181 ± 9	2500	49
10	89 ± 5	3000	60
20	22 ± 2	3500	87
40	0	4000	99
125	77	4500	95
150	48	5000	4
175	42		
200	27		
225	0		

When the threshold was set at 150 mm there was near-perfect agreement between the number of observed whole body displacements and computer counts. Thus, approximately 150 mm is the acutal vector length for computer detection of a between quadrant movement (i.e., gross movements). Thresholds which are increasingly higher than 150 mm produce, for gross movements, more false negatives while lower thresholds produce more false positives.

(c) Demonstration of Evolution of Stereotypy

Figures 4 to 6 show the analog voltages and the associated digital output for three specific behaviors: rears (rear threshold = 250 mv), gross movments (gross movement threshold = 100 mm), and fine movements (fine movement threshold = 5 mm) measured continuously for 60 minutes after an injection of d-amphetamine. Figure 4 shows random hyperactivity with rearing at 5 minutes (note, two rears) leads to more repetitive hyperactivity with more rearing at 10 minutes (note, six rears). By 20 minutes, rearing is absent and by 30 minutes, there is repetitive circling around the cage (note, rhythmic waves) and by 50 minutes movements are in place, restricted and repetitive (note, small amplitude waves only in SE quadrant) (see Figure 6). These data represent the time-dependent qualitative shifts in amphetamine-induced behavior

from generalized excitment to repetitive hyperactivity to repetitive restricted movements.

(d) Supporting Data

Fischer 344/Mai rats (male, 250 g) were used in testing of the system. With thresholds set at 2500 MV for rears, 150 mm for gross movements and 5 mm for fine movements, experiments were performed to:

(1) Demonstrate entrained and free running rhythms;

(2) Differentiate among the acute effects of amphetamine, apomorphine and B-phenylethylamine and;

(3) Trace the effects during a six hour perfusion of apomorphine.

(1) Rhythms

In order to demonstrate the ability of the system for longidutinal measurement, rats were entrained to a 7 A.M. - 7 P.M. light-dark cycle and then placed under 24 hour constant illumination. Under constant illumination, we obtained the expected: (i) response reduction, suggesting an apparent loss of the regular circadian temporal control and (ii) disappearance of 24 hour cycling, replaced by circadian cycling with onset of rearing delayed about 1 hour per day.

(2) Amphetamine, Apomorphine and Phenylethylamine

Rats were administered doses of d-amphetamine, apomorphine or phenylethylamine. Drug injections were preceded by a 30 minute adaptation period and followed by a 120 minute test period. Every 10 minutes the following responses were recorded: number of rears, amount of rear time, gross movements, fine movements and amount of time spent in each quadrant. Several observations can be made from these data: (i) Temporally, the effects of amphetamine proceed through three phases: hyperactivity, defined by increased rears and gross movements and low quadrant variance (10 - 20 minutes); maximal stereotyped behavior, defined by absence of rears and gross movements, increased fine movements and high quadrant variance (30 - 50 minutes); reappearance of hyperactivity with disappearance of stereotyped behavior (100 minutes). (ii) Apomorphine produces effects that are quantitatively and qualitatively different from amphetamine. Initially, apomorphine causes hyperactivity, characterized by a smaller increase in rears and gross movements (relative to amphetamine). Both the peak amphetamine and apomorphine-stereotypy were characterized by increased fine movements and high quadrant variance; they could be differentiated by the absence of rears and gross movements for amphetamine and increased rears, rear time and gross movements for apomorphine. Apomorphine-hyperactivity outlasts stereotypy because at 50 minutes there was still a larger number of gross movements but stereotypy was on the decline. Apomorphine has a smaller effect than amphetamine on stimulating fine movements and gross movements but a greater effect on stimulating rears. (iii) Different from amphetamine and apomorphine, phenylethylamine affects fine movements without altering rears or gross movements. Phenylethylamine produces an increase in fine movements which is about the same order or

less than amphetamine. This effect lasts for 70 minutes at which time most rating scales for stereotyped behavior indicate that phenylethylamine's effects have dissipated.

(3) Apomorphine Perfusion

As a preliminary test of the ability of this system to trace the evolving pattern of behavioral effects caused by chronic drug administration, we used a subcutaneously implanted reservoir. With this technique, drug diffuses out of the reservoir, through a dailysis membrane over one end of the reservoir, at a predictable rate. This allows for maintenance of selected circulating levels of the drug for many hours. The reservoir can be emptied or refilled with fresh drug solutions.

The rat's light-dark cycle was reversed so that the drug could be administered during the animal's active period. A reservoir was subcutaneously implanted in the rat who was then acclimated to the activity cage for two days. The reservoir was filled with 10 mg/ml apomorphine hydrochloride and behavioral responses were recorded for six hours. After six hours, rats were rated as showing maximal stereotyped behavior and apomorphine was emptied from the reservoir for subsequent assay. The fluid removed from the reservior was analyzed to determine the concentration of apomorphine. Despite the facts that every rat was both scored for maximally identical amounts of stereotyped behavior and 80% of the apomorphine was remaining in the reservoir, qualitatively and quantitatively different response profiles were obtained. Among rats given apomorphine the following differences are noticeable: (i) exclusively repetitive increases in fine movements (ii) predominantly repetitive increases in rears, (iii) disassociation between in fine and gross movements.

Applications

We employed a capacitance sensing device and developed algorithms for computer monitoring of different parameters of rat motor activity. This system was developed for the longitudinal measurement of patterns of rat motor activity in studies of chronic drug administration, related to our interest in animal models of neuropsychiatric disturbances.

In addition, our system has wide applications to studies of biological rhythyms with the unique advantage of characterizing the circadian and ultradian, rhythyms of specific motor responses which are mediated by specific neurotransmitters.

Chronic central nervous system stimulant administration in man produces features of naturally occurring psychosis and this human model psychotic can be empirically tested using animal models. The induction of psychotic symptoms in humans (and abnormal behaviors in animal model systems) by central nervous system stimulants is often delayed and neuroleptic drugs have a therapeutic time latency for the alleviation of such symptoms. These time dependent changes are not mere temporal extensions of those seen after acute treatment simply requiring the continued presence of the agent inducing the change, but instead involve a sequence of neurobiological responses associated with qualitative shifts in behavior. This system represents our attempt to characterize in temporal and spatial dimensions the reorganization that occurs in the central nervous system, as reflected in behavior, due

to chronic drug administration. Its applications to sleep, aging and drug toxicology have yet to be explained.

2. Shuttlebox Avoidance

We have conducted a series of investigations in rats of the effects of hallucinogenic drugs in the shuttlebox paradigm. We report that both LSD and mescaline have two distinct opposite effects of the same doses and postulated that one of these effects is analogous to the "good trip" in humans and the other is analogous to the "bad trip". The two distinct effects produced by LSD and mescaline on shuttlebox responding are: (1) excitation or facilitation of responding and (2) inhibition or disruption of responding. The presence of stress is common to both excitation in rats and "bad trip" effects in man, whereas the relative absence of stress is common to both disruption in rats and "good trip" effects in man. Excitation in rats may parallel the "bad trip" effects in man because excitation occurs in a relatively stressful situation and likewise, the "bad trip" effects in man occur in patients who present themselves in hospital emergency rooms with acute psychotic disturbance and emotional stress associated with drug intake. Similarly, disruption in rats may parallel the "good trip" effects in man since disruption occurs in a relatively nonstressful situation and likewise, the "good trip" effects in man occur in a pleasant and supportive environment.

3. Lesion-Rotation Model

Chronic exposure to dopamine agonists occur in two clinical situations: chronic amphetamine abuse and the use of levo-dopa in the treatment of parkinsonism. In both situations, psychiatric disorders and abnormal movements are frequently observed. The supersensitivity phenomonon may be an important factor in the stimulant psychoses and in the induction of dyskinesia in chronic amphetamine users. A quantitative animal model has been developed which has been useful in assessing the potential therapeutic value of drugs in the treatment of parkinsonism. This model may prove useful in developing treatments for drugs which stimulate dopamine because it provides a way of telling whether the drug is acting pre- or post-synaptically. Such pharmacological dissection sheds light on the drug of choice for treatment. It has been shown that drugs which indirectly stimulate dopamine receptors produce turning behavior in unilaterally lesioned rats toward the lesioned side and drugs which directly stimulate dopamine receptors produce turning behavior towards the unlesioned side.

Drugs

1. Speed: Amphetamine

Chronic amphetamine-induced behavioral changes in animals are invariably accompained by motor stereotypies which are species-specific. The significant characteristic of these behavior patterns is the compulsive way in which they are expressed, to the exclusion of other behaviors which are normally present, such as grooming, exploration or feeding. The fact that the particular limited response is performed repeatedly, often rhythmically and apparently without purpose, characterized it as "stereotyped". An extensive and systematic series of studies utilizing such techniques as selective enzyme inhibition, agonist-antagonist relationships, and

local microinjections have documented the importance of central dopaminergic facilitation (particularly in the striatum) in mediating this stereotyped behavior. These studies led to the development of the concept that drug-induced stereotyped behavior in animals might serve as a model for stimulant psychosis in humans, a well-known side effect of chronic amphetamine abuse. The persistent stereotypy in chronic amphetamine addicts has been observed in several different studies. It has been referred to as "punding" by Rylander, G. in Abuse of Central Stimulants, F. Sjoquist and M. Tottie, eds., Raven Press, New York, 1969, who noted that Swedish Preludin (phenmetrazine) addicts were preoccupied with useless, repetitive, minute tasks.

Recent Results

Genetic differences in behavioral response to d-amphetamine have been reported in mice and rats. We have been studying behavioral responses of a number of inbred strains of mice to d-amphetamine and to haloperidol followed 30 minutes later by d-amphetamine. We have tested 11 strains of mice, including two parent strains and seven recombinant inbred strains. We have studied the stereotypy and locomotor responses of these strains. The tests with inbred strains in mice are useful for delineating genetic transmission of responsiveness to drugs. Our results so far have interesting implications for understanding possible hereditary transmission of responses to d-amphetamine and haloperidol. During the past year we have extended this work to β-phenylethylamine. We are employing also, the shuttlebox paradigm to characterize the response to amphetamine and β-phenylethylamine among the recombinant inbred strains.

2. The Natural Speed: Phenylethylamine

Phenylethylamine is almost structurally identical to amphetamine and has similar pharmacological, physiological and behavioral actions. Since phenylethylamine is amphetamine-like, but is naturally present, whereas amphetamine is not, phenylethylamine may serve as a useful animal model to study chronic stimulant abuse and the accompanying residual effects. The psychoses which outlasts the presence of amphetamine in urine occur for several months following the cessation of amphetamine use. The persistent psychotic states may be due to the continued persence of a naturally occurring amphetamine such as phenylethylamine.

In a series of experiments, we have shown that phenylethylamine has amphetamine-like behavioral effects when monoamine oxidase is inhibited. These effects are antagonized by dopamine receptor blockers and testosterone. Chronic neuroleptics appear to cause supersensitivity to phenylethylamine. Tolerance develops to phenylethylamine's effects on learned behavior, but not to phenylethylamine-induced stereotypy. We found that two other compounds have similar behavioral effects to amphetamine and phenylethylamine: paramethoxyphenylethylamine, a derivative to phenylethylamine and amfonelic acid, a specific central dopaminergic stimulant. The serotonergic system appears to be involved in the behavioral stimulating effects of mescaline and amphetamine.

Recent Results

Catalepsy is the pharmacological converse of stereotypy. Drugs which induce catalepsy are capable of antagonizing stereotyped behavior. As a model of protection against the stimulant psychoses, we have studied whether central nervous system stimulants reverse chlorpromazine's catalepsy and allosteric activation of caudate tyrosine hydroxylase. We found that phenylethylamine, amphetamine, and apomorphine cause dose-dependent reversal of both chlorpromazine catalepsy and tyrosine hydroxylase activation.

The repeated administration of phenylethylamine to laboratory animals produces neither tolerance nor physical dependence. "Reverse tolerance" has been used to describe the increased susceptibility to the stimulant effects of phenylethylamine. As a model of the persistent effects of stimulants with long-term use, we have studied the effects of chronic phenylethylamine on rats to tyrosine hydroxylase activity. A dosage of 600 mg/kg, 14 times, produced behavioral stimulation lasting 5-7 hours per day. Phenylethylamine caused a 20% increase in the Vmax of striatal tyrosine hydroxylase which outlasted the duration of chronic phenylethylamine treatment. Concurrent administration of haloperidol (1 mg/kg, ip) did not influence the stimulation of striatal tyrosine hydroxylase by chronic phenylethylamine, suggesting that the effect is independent of dopamine-receptor stimulation. Furthermore, this effect does not occur in substantia nigra or locus coeruleus suggesting it is not a general effect on tyrosine hydroxylase. Others have reported that chronic administration of cocaine, like phenylethylamine, also stimulates striatal tyrosine hydroxylase. Thus, the "reverse tolerance" of phenylethylamine and cocaine may both be related by increased synthesis of dopamine and norepinephrine. Synthesis inhibitors of dopamine and norepinephrine may be effective antidotes against long term effects of phenylethylamine and cocaine.

3. The Businessman's Trip: N,N-Dimethyltryptamine

Because dimethyltryptamine is a short-acting hallucinogen, it gained the reputation of its nickname as a reprieve from the hard working day. Dimethyltryptamine is found in some human tissue. Enzymes for producing it are present in human lung and may be present in human brain and blood as well. We found that when dimethyltryptamine is given to man it produces an intense psychological disturbance. Because of the brevity of its effects and the intensity of it reaction, it has become well known in the drug subculture.

To discover an effective antidote to dimethyltryptamine, we have conducted an extensive series of drug interaction experiments using the n, n-dimethyltryptamine paradigm. Dimethyltryptamine disruption is not blocked by acute or chronic pretreatment with drugs which inhibit serotonin, dopamine, or norepinephrine. These data provide evidence against a monoaminergic mechanism of dimethyltryptamine suggesting that it acts nonspecifically or through its own, as yet undisovered, receptor.

4. Angel Dust: Phencyclidine

Misrepresentation of phencyclidine is widespread because of its availability. Users are often led to believe that they are being sold more exotic natural drugs, such

as mescaline, when in fact the pills are entirely phencyclidine. Such deception makes it difficult to make an accurate diagnosis and provide the best treatment. Individuals who have taken phencyclidine do not describe the increased ability for self-observation or kaleidoscope visual hallucinations reported by users of the more classic hallucinogens. On the contrary the phenycyclidine experience can be unpleasant with impaired thought, disorientation, delirium and marked anxiety. There is some concern that phenycyclidine intoxication leads to behavioral changes persisting for days or weeks after drug use.

We have tried to develop an animal model for persistent effects of phencyclidine. We found that phencyclidine facilitates acquisition of shuttlebox avoidance and that this effect may last at least 24 hours after drug injection. Further, this effect may be state-dependent which has been suggested as a predictor of the abuse potential of drugs.

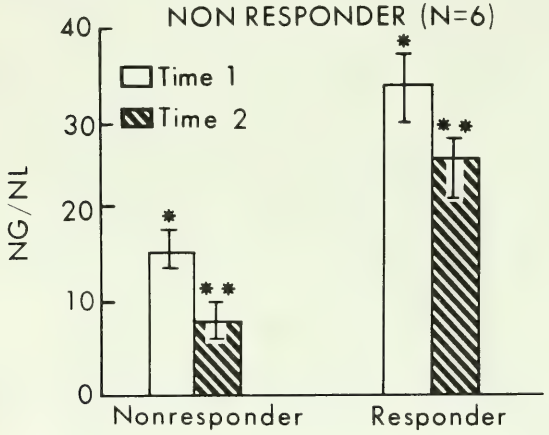
Phencyclidine is known to produce a dose-related stimulant of locomotor activity in mice similar to the activation produced by amphetamine. We have used this property of phencyclidine as an animal model to screen for agents capable of blocking behavioral effects of phencyclidine. Because many drugs influence the locomotor activity of animals, phencyclidine-induced stimulation was evaluated in relationship to the effects of the drugs on the locomotor activity in the absence of phencyclidine. Drugs which were found to be capable of completely blocking this effect of phencyclidine included chlorpromazine, clozapine, muscimol, imidazole-4-acetic acid, and methylsergide in high doses. Baclofen, cyproheptadine, haloperidol and yohimbine were partially effective. Many other agents, such as naloxone, diazepam, cinnanserin, propranolol, benztropine, arecoline, pilocarpine, reserpine, alpha-methylpara-tyrosine, phentolamine, diphehydramine, and coloral hydrate were either ineffective or enhanced effects of phencyclidine. Neurochemical studies suggest that phencyclidine interacts primarily with brain cholinergic and opiate receptors, although effects on metabolism of serotonin and norepinephrine have also been described. The present results suggest that manipulations of brain dopamine, serotonin, and gamma-aminobutyric acid are most effective in blocking phencyclidine-induced behavioral alterations. It is therefore concluded that studies of neurotransmitter binding and metabolism are not good predictors of the neuropharmacology of phencyclidine-induced alterations in behavior.

Publications

Freed, W., Weinberger, D., Bing, L., and Wyatt, R. J.: Neuropharmacological studies of phencyclidine (PCP)-induced behavioral stimulation in mice. Psychopharmacology 71:291-299, 1980.

Parker, E. S., Birnbaum, I. M., Weingartner, H., Hartley, J. T., Stillman, R. C., and Wyatt, R. J.: Retrograde enhancement of human memory with alcohol. Psychopharmacology (in press).

MEAN SALIVA BENZODIAZEPINE CONCENTRATION AT TIMES 1 AND 2 FOR RESPONDER (N=12) AND NON RESPONDER (N=6)



* T (time 1, responder -vs- nonresponder) =1.75, p=0.1

** T (time 2, responder -vs- nonresponder) =2.53, p=0.02

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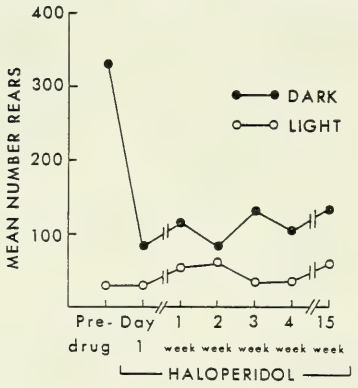

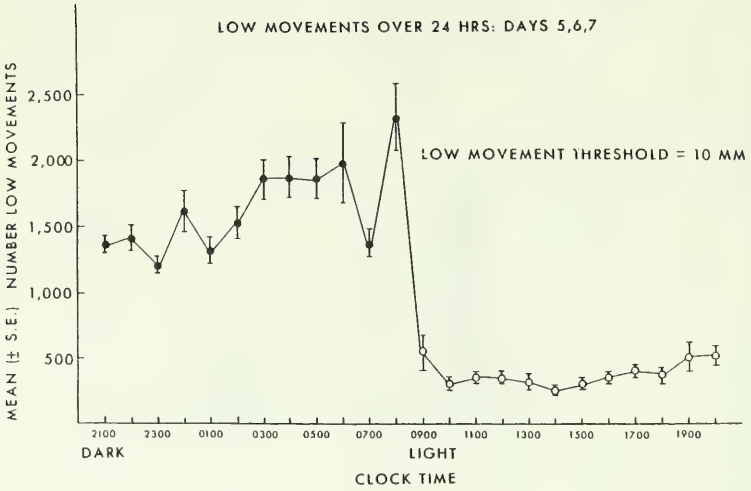
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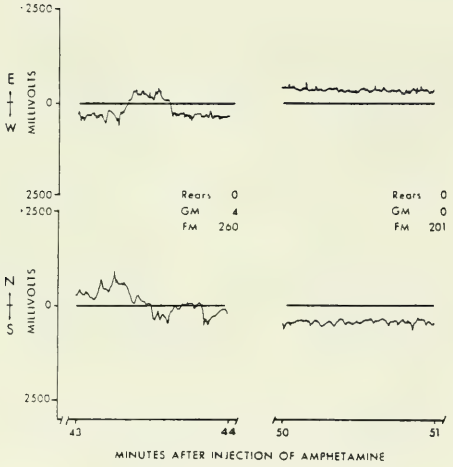
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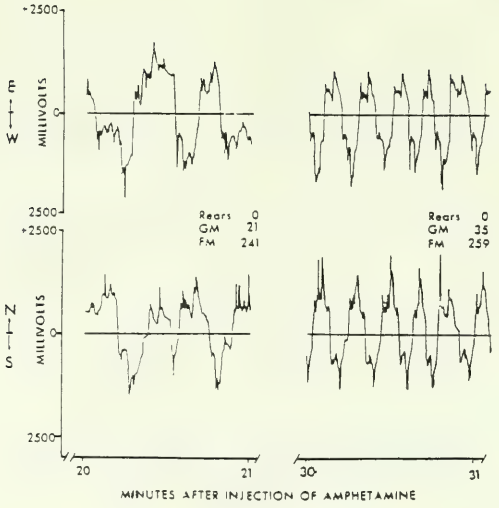
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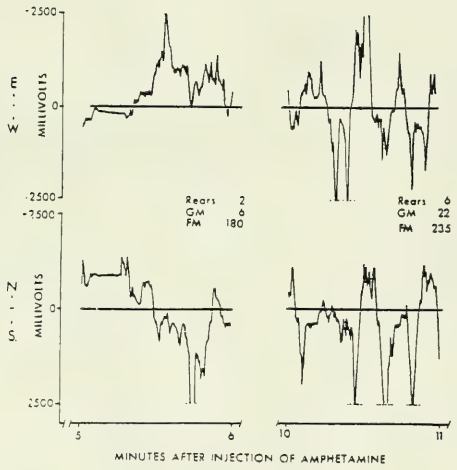
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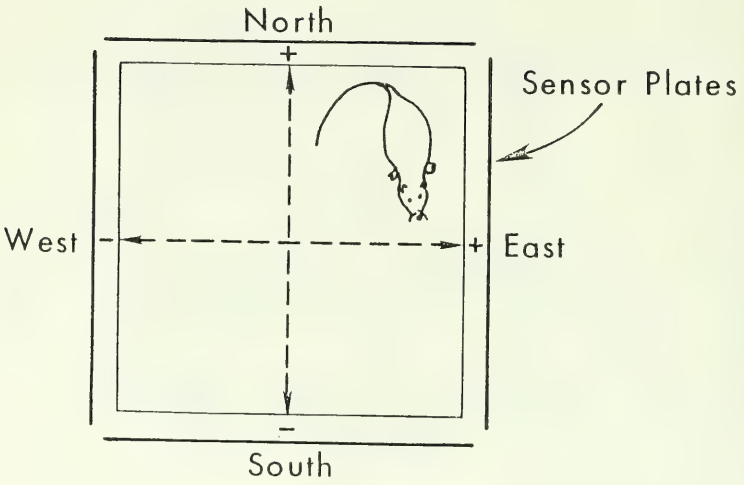
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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01338-03 SMRA

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R.J. Wyatt, Chief, Adult Psychiatry Branch, SMR; J.C. Gillin, Deputy Chief, APB, SMR; Staff Psychiatrists, APB, SMR, W.B. Mendelson, D. Shore, D.R. Weinberger, T.P. Bridge; Staff Fellows, APB, SMR, B. Phelps, L.M. Neckers, A. Fine, A. Church, W. Watson, W.J. Freed; Visiting Associate, APB, SMR, L. DeMedinacelli; Staff Psychiatrists, APB, SMR, D.V. Jeste and N. Cutler; Staff Fellow, APB, SMR, A. Sostek

OTHER: J. Brodie, NIA; E. Parker, NIAAA; S. Rapport, NIA; B.J. Hoffer, University of Colorado; L. Olsen, Karolinska Institute; A. Seiger, Karolinska Institute; C. Gajdusek, NINCDS; H. Weingartner, DCBR; M. Ebert, DCBR.

COOPERATING UNITS (if any)

National Institute of Neurological Disease, National Institute of Aging, National Institute on Alcohol and Drug Abuse, Karolinska Institute, University of Colorado

LAB/BRANCH
Adult Psychiatry Branch

SECTION
Unit on Geriatric Psychiatry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12	8	4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The following report is an overview of the research studies initiated and the up-date of on-going studies by the Unit on Geriatric Psychiatry for 1980. The research projects have been catagorized as follows:

I. Metabolic Disturbance
II. Cognitive Performance
III. Nerve Repair
IV. Neuroleptics
V. Senile Dementia
VI. Parkinson's Disease
VII. Sleep Studies

Please note, however, that these catagorizations are made. The hypotheses being tested methodologies employed and implications of results often overlap. This is the natural concomitant of a broadly based research program designed to pursue questions concerning the natural processes and pathologies of aging.

Project Description:

Objectives:

The objectives for research in the Unit of Geriatric Psychiatry are to test existing hypotheses and create new hypotheses relating to the social, psychological, cognitive and affective changes that occur through the aging process. Further, it is our objective to perform research that illuminates the differences between normal aging and pathology, synthesizing work from specific disciplines as well as interdisciplinary efforts.

I. Metabolic Disturbance

A. Platelet Monoamine Oxidase

Observations have been made previously that platelet, plasma and brain tissue monoamine oxidase activity is elevated in older individuals relative to younger subjects. This evident increase in enzyme activity with advancing age is in contrast to the other catecholamine enzymes. In addition to advancing age, certain pathologic states have been associated with elevated platelet monoamine oxidase activity including depressive disorders, senile dementia, and glaucoma.

Population studies of other catecholamine enzymes have demonstrated that very low enzyme activity is under genetic control, probably inherited as an autosomal recessive trait. Susceptability to heat inactivation (thermolability) believed to be one of the most sensitive indicators of altered enzyme amino acid sequence, is under the control of the structural gene for the enzyme. Very low thermolability, which correlates with low enzyme activity in the general population samples is also evidently inherited as an autosomal recessive trait within families where both traits are present. Thermolability and low enzyme activity, however, sort independently for these other catecholamine enzymes.

Data are reported from a general population study (N=237) of platelet monoamine oxidase activity in a demographically diverse sample without evidence of psychiatric illness (often associated with altered platelet monoamine oxidase activity). Within the entire sample, age correlates positively with enzyme activity (r=.49). No such relationship is present, however, in those subjects age 18-50 or those aged 51-79. The decade 50-59 shows the bulk of the increment in enzyme activity. In older individuals in this sample (age 50) there is a phenomenon of increased thermolability relative to younger subjects. This finding in the older subjects is opposite to the usual association of low enzyme activity and thermolability (Figure 1).

The total number of subjects is being increased in order to identify a subset of subjects with heat sensitive platelet monoamine oxidase. With the full compliment of subjects, data from kinships with heat sensitive monoamine oxidase will be used to identify the mode of inheritance of this trait. We hope that normative data collected from this research will aide us in understanding the extent of these changes as well as identify potential genetic interplay with age effects in reference to platelet monoamine oxidase.

In related work with separate populations of older schizophrenic patients (N=27), nursing home residents (N=15) without evidence of functional psychopathology, and elderly residing in the community (N=43), activity of two catecholamine enzymes, monoamine oxidase and dopamine-beta-hydroxylase were measured in platelets and plasma respectively. Separate assessments of functional psychopathology by the Research Diagnostic Criteria and cognitive function by the Folstein Mini-Mental State Exam were made by board certified psychiatrists blind to individual subjects' enzyme activity. Data are presented demonstrating age dependent relationship of enzyme activity and cognitive function. Within these three groups, evidence is found for distinct patterns of enzyme activity for both functional psychopathology and cognitive function.

These results showed that as platelet monoamine oxidase activity in both groups increased, cognitive performance on the Folstein instrument worsened. There was also a trend for similar indirect relationship between serum dopamine-beta-hydroxylase and Folstein scores. These results tend to support previous work in this area and to point to a relationship of the dopaminergic system in cognitive impairment of the elderly in general, and in older schizophrenics in particular.

Further, we are assessing data contained in the Health Inventory Survey for diseases that have been reported to be associated with alterations of platelet monoamine oxidase. Particularly, we are examining these data in terms of decreased activity as it relates to schizophrenia, diabetes and alcoholism and increased activity as it relates to dementia, Parkinson's disease, glaucoma and depression.

Data is compiled on all members of a household within 50,000 households. The data identified by kinship presents the opportunity to observe any potential relationship between the prevalence of these diseases within the kinships relative to the prevalence of these diseases within the general population.

B. Renal Dopamine Receptors In Hypertension

Many lines of evidence suggest that dopamine has a physiological role in the kidney. It has been demonstrated that there are dopamine-containing neuronal elements in the glomerular vascular poles of canine kidney. Also demonstrated are dopamine-specific receptors in renal arteries mediating the vasodilation produced by exogenous dopamine. The involvement of dopamine in regulating renal sodium and renin excretion also has been reported.

The possible pathological significance of a peripheral vascular dopamine receptor is suggested by the potent pressor effects of dopamine in human subjects and the finding of abnormalities in renal blood flow in the offspring of hypertensive parents. Since dopamine appears to be involved in regulating renal blood flow, and since abnormalities in renal blood flow have been associated with essential hypertension, we studied the binding of the dopamine receptor ligand ^{3}H-spiroperidol in kidneys of normotensive and spontaneously hypertensive rats.

The kidneys of one hundred fifty spontaneously hypertensive rats and their normotensive strain mates were excised, the fibrous renal capsules stripped and the kidneys were bisected sagitally. The hemi-kidneys were then sectioned into a cortical

strip, medullary strip, and renal pelvis. Immediately after dissection, the tissue was homogenized for centrifuging and further analysis.

The stereospecific binding of ^{3}H-spiroperidol, defined as the amount bound in the presence of levo-butaclamol minus the amount bound in the presence of dextro-butacolamol, was 30% to 40% of the total binding. A Scatchard analysis of the specific binding gave a straight line indicating a single population of non-interacting sites.

In order to determine whether ^{3}H-spiroperidol binding in renal cortex had the same specificity for dopaminergic agonists and antagonists as reported for brain, ^{3}H-spiroperidol binding in kidney and brain was assayed in parallel experiments in the presence of various concentrations of several different unlabeled compounds. The order of potency of unlabeled neuroleptics in displacing ^{3}H-spiroperidol was similar in kidney and brain; pimozide was the most potent in both kidney and brain followed by fluphenazine, haloperidol and chlorpromazine. The alpha receptor antagonist phentol-amine was more potent in kidney than in caudate. Interestingly, dopamine and the dopamine partial agonist apomorphine were considerably less potent than dopaminergic antagonists.

The distribution of ^{3}H-spiroperidol stereospecific binding varied among the three major regions of the kidney. Following incubation, more stereospecific binding was observed in renal cortex than in renal medulla, while no stereospecific binding was observed in renal pelvis.

The binding characteristics of ^{3}H-spiroperidol in rat renal cortex indicate stereospecific binding to a high affinity neuroleptic site. The order of potency of different neuroleptics in displacing ^{3}H-spiroperidol was the same for renal cortex and caudate with clinically and behaviorally more potent neuroleptics in displacing stereospecific ^{3}H-spiroperidol binding. The lower affinities of dopaminergic agonists in inhibiting ^{3}H-spiroperidol binding in renal cortex as compared with caudate, as well as the somewhat greater affinities of phentolamine in renal cortex, suggest that renal cortical and caudate binding sites may be different. Interestingly, chronic haloperidol treatment had no effect on renal cortical ^{3}H-spiroperidol binding, suggesting also that renal cortical and caudate neuroleptic sites are different.

Renal dopamine receptors appear to be involved in regulating renal blood flow, tubular sodium transport, and renin excretion, physiologic parameters known to be associated with some forms of hypertension. Our finding of increased neuroleptic receptor binding in spontaneously hypertensive rats suggests that some forms of hypertension in humans may be associated with abnormalities of renal dopamine receptors.

C. Flurazepam

The metabolic fate of flurazepam has been studied in animals and humans by using radioactive tracer techniques, spectrophotofluorometry and gas chromatographic techniques. Most of these studies described the pharmacokinetics of flurazepam in plasma or urine samples and indicate that N_1-desalkyl flurazepam is slowly biotrans formed and accumulated during chronic therapy. Because the N_1-unsubstituted

metabolite of this drug is pharmacologically active, it may be responsible for the unwanted toxic effects of flurazepam in the elderly following a 30 mg per night dose regime. The present experiments were designed to study the accumulation of lipophilic metabolites in the central nervous system of the cat after i.v. administration of 30 mg/kg of flurazepam. A gas chromatographic-mass spectrometric procedure is used in order to identify flurazepam and its metabolites in cerebrospinal fluid and plasma samples. A positive identification of benzodiazepines by mass spectrometry requires at least 50 to 100 ng per injection of sample into the Hewlett-Packard-5992 gas chromatographic-mass spectrometric system. Results confirm that the major metabolite of flurazepam in the blood was the N_1-desalkyl analogue, which accumulated significantly up to 24 hr after administration of a single dose of flurazepam. A positive identification of unchanged and N_1-desalkyl flurazepam in the cerebrospinal fluid was obtained 60 min after i.v. administration of the drug. There was an accumulation of this N_1-unsubstituted metabolite ins the cerebrospinal fluid 24 hr after a single dose of flurazepam. The half life of flurazepam in plasma and cerebrospinal fluid appeared to be less than 2 hr. In summary, after a single i.v. administration of flurazepam, the unchanged analogue disappears quickly in plasma and cerebrospinal fluid while the N_1-desalkyl metabolite is slowly accumulated in blood and cerebrospinal fluid.

D. Hepatic Enzyme Induction And Age

It has been shown in a variety of studies that in general hospitals there is a much higher incidence of side effects requiring discontinuence of medication in elderly patients. This is true even when medicine which tend to be prescribed in the elderly and are particularly toxic, such as digitalis, are removed from the data. There may be several possible reasons for such an increased incidence in side effects in the elderly, including impaired hepatic metabolism of drugs, altered volume of distribution, or possibly altered end organ sensitivity. In the case of psychoactive drugs, the end organ sensitivity of course translates into the issue of whether the brain is some how more sensitive to drugs in elderly individuals. Previous studies have indicated that in some cases this may be true. It has been noted, for instance, that acetylcholine applied to the cortex of young rabbits produces only local spiking, whereas in elderly rabbits spiking generalizes over most of the surface of the cortex.

In those cases in which altered hepatic metabolism may explain increased side effects in the elderly, it may be possible to actually improve the rate of metabolism of drugs by use of enzyme induction techniques. It is well known that a number of agents, such as the barbiturates induce enzymes that metabolize many drugs. This, however, has traditionally been considered an unwanted side effect. On the other hand, if a barbiturate derivative could be found, which in itself does not have significant psychoactive properties (i.e., it is not sedating, would not cause hypo tension, etc.) it might be considered beneficial to have such a drug improve hepatic performance when a person is exposed to other drugs. In looking into this question we have found several barbiturate derivatives, most importantly one known as pyrictal, which are reputed to have little psychoactive properties yet to be potent enzyme inducers. It is our plan to confirm both of these qualities, first to determine whether it has psychoactive properties and second if this turns out not to be the case, then to determine its potency in inducing enzymes. As a model system we planned to examine the rate of break down of ethyl morphine. We plan to compare the basal rate of break

down of ethyl morphine in young and old rats and then to determine whether the use of pretreatment by pyrictal will: a) lower the toxicity of ethyl morphine and; b) cause a more rapid break down of this drug. In addition to measuring alterations in rate of break down of ethyl morphine we plan to look, also, at the increase in hepatic cyctochrome p450 in both young and old rats. It is our hope that if this technique proves useful, it can be applied to other classes of agents, possibly becoming useful as a tool for treatment of toxicity of other drugs.

II. Cognitive Performance

A. Amino Acid Antagonists

There is some evidence that glutamate plays an important role in the mammalian brain as an excitatory neurotransmitter. Glutamate is found in high concentrations in the hippocampus and cortex and may be the transmitter for excitatory hippocampal afferents and the corticostriatal pathway. Glutamate is capable of exciting most, if not all, neurons and is released both from the brain and peripheral nerves in association with activity. It has been suggested that patterned release of glutamate occurs in localized areas of the brain during learning, and that this phenomenon produces a patterned facilitation of synaptic transmission that facilitates, or forms the basis for, learning.

Glutamic acid diethyl ester is a partially effective antagonist of glutamate-induced neuronal excitations. This chemical has been found to be particularly effective in the hippocampus, where it inhibits the action of glutamate and dampens evoked field potentials. It has been found, also, to competitively inhibit the binding of glutamate to its putative membrane receptor. The relative effectiveness of gluatmic acid diethyl ester in the hippocampus, a brain area that has an important role in some forms of learning, suggested to us that this chemical might be capable to interfering with learning. The purpose of the present study, therefore, was to determine whether glutamic acid diethyl ester would impair instrumental learning in rats.

Thirty-two male Sprague-Dawley albino rats were chosen. Testing was conducted in four similar chambers. L-glutamic acid diethyl ester was administered with the highest concentration dissolved in distilled water and diluted with normal saline. Control animals received normal saline.

At the start of the testing, the chamber bars were disconnected and food pellets were delivered noncontingently. The next day one of the bars, either the right or the left, was activated so that each bar press resulted in the delivery of one food pellet (correct bar). The other bar remained inactive (incorrect bar). The position of the correct and incorrect bars remained the same for each particular animal for all phases of testing.

Two days after the learning test, the animals were again placed in the chambers. No noncontingent food pellets were delivered and no drugs were administered. After a two week rest period, the animal's performance was retested.

The results of this study found that glutamic acid diethyl ester impaired acquisition of the bar-press response in that the two highest doses essentially prevented learning. Total number of correct (reinforced) responses were decreased, while the number of incorrect (unreinforced) responses was unchanged. In addition, the time required for the animals to reach a criterion of 90% correct responses was greatly increased by glutamic acid diethyl ester, and the mean percentage of responses that were incorrect was increased by about 40% by the two highest doses.

Table 1

EFFECTS OF GLUTAMIC ACID DIETHYL ESTER (GDEE) ON SEVERAL MEASURES (MEAN ± SEM) OF LEARNING

	Dose (mg/kg)					
Measure	0	120	240	480	F(df)	P
Time to obtain 100 food pellets (min)	71 ± 16	56 ± 6.8	125 ± 22	135 ± 22	4.85 (3,28)	.008
Time to achieve 90% correct responses overall (min)	90. ± 23	68 ± 25	145 ± 23	170. ± 10.	5.03 (3,28)	.007
Time to the first 10-min. interval with 90% correct responses (min)	50. ± 13	25 ± 3	120. ± 27	124 ± 27	6.19 (3,28)	.003
Total number of correct responses	182 ± 9.0	181 ± 14.0	96. ± 35.2	77. ± 27.4	5.39 (3,28)	.005
Total number of incorrect responses	21 ± 9.1	21 ± 7.5	28 ± 10.8	50. ± 25	0.65 (3,28)	.519
Percentage of total responses that were incorrect	8.6 ± 3.1	9.4 ± 3.0	41.9 ± 11.5	40.2 ± 7.0	6.88 (3,28)	.002

Recall: Latency of first correct response (sec.)	67 ± 20.	76 ± 21	138 ± 65	586 ± 344	2.90 (3,23)	.056
Recall: Latency of 10 correct responses (sec)	174 ± 33	180. ± 28	616 ± 201	879 ± 377	3.21 (3,23)	.041
Recall: Correct responses in the first 10 min	43 ± 3.6	36 ± 2.7	24 ± 8.2	18 ± 5.1	4.32 (3,27)	.013
Recall: Correct responses from 10 to 30 min	66 ± 5.5	52 ± 5.9	52 ± 122	42 ± 12.2	1.13 (3,27)	.355

B. Attention and Memory

The cognitive performance of older persons appear to be hampered by decreased memory and increased attentional deficits. It is held, generally, that the ability to perform these tasks in the human information processing system deteriorates throughout the normal aging process. It is observed, further, that older persons have difficulty ignoring irrelevant information. Information that is not needed but is not ignored hinders performance by occupying the processing resources that are already at a premium in the older person. In addition to older persons, schizophrenics exhibit similar performance deficits and are being studied, also, within this context.

The aim of our work in the role of irrelevant information is to investigate whether or not the problem is non-specific or related to specific characteristics of the irrelevant information. Non-specific is defined here as occurring for all types of distractors. Specific characteristics apply to such categories as response competition, category effects and masking.

Populations of older persons and schizophrenics are assessed on performance of divided attention tasks. Subjects are given a main task onto which a secondary task is added. An example of such a task sequence is that a subject is given a set of letters to memorize. Subsequently, the subject is directed to view a letter on a screen which is flanked by irrelevant letters. The subject is to determine if the center letter is one previously memorized. This study is, presently, in the initial data collection phase.

III. Nerve Repair

It is considered axiomatic that complete severance of a peripheral nerve results in an absence of conduction across the gap, even if the nerve is repaired. Regardless of the method of repair, impulses initiated in the proximal stump of a severed nerve

have not been recorded in the distal stump prior to restoration of the continuity of the fibers by regeneration. The purpose of our work is to determine if, and under what conditions, conduction can be obtained following reconnection of the stumps of a freshly transected peripheral nerve.

Peripheral nerve regrowth after injury is hampered by the development of scar formation and interposition of fibrous tissue. When the scar formation is reduced to a minimum, sprouts can cross with greater ease.

We are developing a technique, using the sciatic nerve of rats and rabbits, to reconnect the recently severed nerve end to its stump. Although true fusion of the severed axons has not been perfected, in certain procedures the tips of certain axons come close to contact. The approximation has been sufficiently close to allow transmission of evoked action potentials across the gap.

While obtaining true fusion is the objective of the present study, work is projected to apply this technique of nerve repair to paraphlegic rats.

IV. Neuroleptics

A. Tardive Dyskinesia

Tardive dyskinesia is considered to be a major complication of long term neuroleptic treatment. Although the introduction of neuroleptics in the mid-1950's is believed to have played a major role in the dramatic decline in the numbers of hospitalized schizophrenics, the overall prevalence of tardive dyskinesia has increased since that time to its present and serious level. It is more likely to occur in the elderly on neuroleptic treatment.

Our work in the area of tardive dyskinesia is being conducted to investigate:

(1) Clinical differences, in terms of epidemiological variables, history of psychiatric illness and its treatment, physical status, blood-neuroleptic level and platelet monoamine oxidase activities, organic brain damage as judged by psychological testing and CAT (computerized axial tomography) scan between dyskinesia and non-dyskinesia patients.

(2) If criteria can be determined to categorize patients into subgroups of risk/no risk patients.

(3) Potential side effects of specific neuroleptic drugs (e.g., thioridazine) and

(4) The relationship, if any, of neuroleptic treatment and retinitis pigmentosa.

After subject selection and consent procedures are completed, data is collected by means of physical and neurological examinations, including blood tests and CAT scan. Patients have follow up checks at regular intervals for up to four (4) years.

To date, the data have yielded the following results:

(1) Tardive dyskinesia is sigificantly correlated with high blood serum levels of neuroleptic drugs,

(2) There appears to be no significant correlation between tardive dyskinesia and obvious structural brain abnormalities, and

(3) The toxic side effect of retinitis pigmentosa has not been found to be associated with the administration of thioridazine in this population.

Another direction we have taken is to study the prevention of, and susceptability to tardive dyskinesia in an animal model. The model demonstrates behavioral supersensitivity to amphetamine following long-term oral administration of haloperidol to rats.

The results of investigating this model indicate that:

(1) Intermittent administration of haloperidol does not retard development of supersensitivity and

(2) Initial low responders to amphetamine develop greater percentage increase in activity counts in response to amphetamine following chronic haloperidol treatment than do initial high responders.

These findings suggest two clinically important and testable hypotheses:

(1) Intermittent neuroleptic treatment may not prevent tardive dyskinesia, and

(2) Initial amphetamine response may predict susceptability to tardive dyskinesia.

To begin testing these hypotheses, research has begun with eleven specially inbred strains of mice. We are examining these strains' baseline locomotor activity learning in two-way conditioned, avoidance shuttle boxes and the effects of the neuroleptics d-amphetamine, phenylethylamine and haloperidol on these behaviors.

Our findings to date show that certain strains respond maximally to the drugs while other strains are non-responders.

In future, we plan to continue study of this differential stain response as well as continuing our studies using drugs to test the effects of long-term administration.

B. Desipramine In Elderly Women

It is generally accepted that there is a higher incidence of adverse reactions of psychoactive drugs in the elderly. Possibly, major mechanisms include increases in free drug concentration per unit dose or altered receptor response. Standard doses of tricyclic antidepressants in elderly patients produce a high incidence of side effects. Standard doses of imipramine produce, also, higher plasma concentrations in depressed

elderly patients than in middle and younger age groups. It is suggested, therefore, that pharmacokinetic factors account for any observed increase of side effects in the elderly. The parallel issue of whether there are age-dependent changes in therapeutic responses cannot be ascertained until the relationship between plasma concentration and therapeutic effect has been defined for discrete age groups.

In order to further explore these questions, we instituted a pharmacokinetic and clinical study of desipramine in a group of subjects who had a mean age of 84.3 years. The expectation was that any age related alterations in pharmacokinetics might be pronounced in this population. Therefore, a single dose prediction of ultimate steady state was used to insure that plasma concentrations would fall within the proposed therapeutic range (40-160 ng/ml).

Subjects were seven female patients, 69-92 years of age, residing in a metropolitan Washington, D.C. nursing home. All subjects had been diagnosed with major behavioral disorders and volunteered to participate in the study as part of their clinical treatment. All subjects had normal liver, thyroid and hematological indicies and were free of psychotropic drugs.

Each patient received an initial dose of desipramine. Blood samples were drawn at 12,24 and 48 hours after drug administration. Following the 48 hour samples, a low maintenance dose of desipramine was begun. After patients had received a fixed dose of desipramine for two to three weeks, blood samples were obtained to determine steady state concentrations.

The results of our work show that even with a limited number of subjects, the prediction of desipramine steady state plasma concentration was accurate. Our results are surprising since we expected that these patients would achieve higher plasma concentrations than those found in our previously studied group of younger patients. Instead, the reverse occurred. The results demonstrate further the applicability of single-dose predictions of steady state concentrations in a non-research setting. In this particular group there were no significant side effects.

Thus, the results in this small number of patients suggest that at least in the case of desipramine, aging does not appreciably alter pharmacokinetics. This may have great practical significance in terms of selecting tricyclic antidepressants for treatment of depression in the elderly.

C. Depressive Symptoms, Cognitive Function And Nortryptyline

To date, few studies have been performed which look at the use of conventional anti-depressant drugs among the elderly. A similar dearth exists for research simultaneously considering the symptomotology of depression and cognitive impairment in elderly persons being treated for depression.

In our study we are administering conventional anti-depressant medication, nortryptyline, to a group of depressed elderly and to monitor the effects. This is a pilot study observing the effects of variant medication levels as reflected in serum and saliva blood levels with respect to behavioral effects.

Patients are being evaluated at the beginning and conclusion of the study. The Folstein Mini-Mental State Exam is being used to determine cognitive functional level at both the pre-test and post-test phases. Patients are being seen weekly to monitor behavioral symptomotology and to measure serum and saliva blood levels. We are measuring the adequancy of nortryptyline uptake and its relationship to behavioral symptomotology and cogntive function.

D. Serum Neuroleptic Concentration And Clinical Response

A sensitive radioreceptor assay for neuroleptic drugs in serum was used to examine the relationship between serum neuroleptic concentration and clinical response. Validity was suggested by close correlation of serum haloperidol concentration measured by both radioreceptor assay and radioimmunoassay. Higher values however, were obtained with the radioreceptor assay, suggesting the possible presence of active haloperidol metabolites.

Repeated freezing and thawing of serum lowered serum neuroleptic concentration by 40-50%. Brief exposure to rubber vacuum tube stoppers, however, and storage of serum in glass did not affect serum neuroleptic concentration.

In a group of 24 haloperidol treated patients, daily dosage was correlated with haloperidol serum concentration. In another group of patients treated with several different neuroleptics, daily dosage was not correlated with serum concentration (in chlorpromazine equivalents) for the group as a whole; however, in subgroups of patients receiving the same neuroleptic, daily dosage was closely correlated with serum concentration. Ratios of serum neuroleptic concentration (in chlorpromazine equivalents) to daily dosage (in chlorpromazine clinical equivalents) differed for different neuroleptics. Serum and CSF neuroleptic concentration were significantly correlated as reported previously. Serum neuroleptic concentration and plasma prolactin were also closely correlated.

There was a significant negative correlation between age and neuroleptic serum concentration. Despite lower daily dosages, older patients had serum neuroleptic concentrations as high, and in many cases, higher than patients under 45 years of age.

In 10 patients at the onset of extrapyramidal reactions, neuroleptic serum levels were relatively low. Seven out of the 10 patients had serum neuroleptic concentrations below 100 ng/ml chlorpromazine equivalents. Also, despite similar neuroleptic dosage, patients treated conjointly with benztropine mesylate (2 mg p.o. b.i.d) had sigificantly lower neuroleptic serum levels. Unlike patients with EPS, patients with tardive dyskinesia were found to have significantly higher serum neuroleptic concentrations than control patients matched for age, weight, drug, dosage, length of neuroleptic treatment, and diagnosis.

In 14 chronic schizophrenic patients at a fixed neuroleptic dosage for at least 14 days, anxiety/depression and thought disorder clusters of the Brief Psychiatric Rating Scale (BPRS) were negatively correlated with serum neuroleptic concentration. In the same group of patients, however, daily neuroleptic dosage was also negatively correlated with the same two symptom clusters. None of the other BPRS clusters, including anergy, activation, hostility, or global response was significantly correlated

with either neuroleptic dosage or serum level. Both anxiety/depression and thought disorder ratings were significantly higher in patients with serum levels below 150 ng/ml (a threshold serum level found for appearance of detectable CSF neuroleptic).

V. Senile Dementia

A. Aluminum, Floride And Alzheimer's Disease

The most common cause of "senility" in the elderly is a degenerative brain disease called Alzheimer's disease or Alzheimer-type senile dementia. This illness is characterized by a progressive decline in memory and intellect, and a deterioration of social, occupational and communication skills. It is estimated that three to five percent of the United States population over age 65 are afflicted with this disease. Many more Americans will be afflicted as the average age of our population continues to rise. There is, presently, no effective treatment for Alzheimer's disease.

In recent years, patients with Alzheimer's disease have been found to have accumulations of aluminum in the hippocampus and cortex of the brain. These accumulations are localized within the nuclei of those nerve cells showing the neurofibrillary degenerative changes typical of Alzheimer's disease. This degeneration is most commonly seen in areas of the brain that are associated, generally, with memory and higher mental functions.

When certain aluminum salts are injected into susceptible animals, aluminum accumulates on DNA in the nucleus within nerve cells and neurofibrillary degeneration and learning impairments are seen. Aluminum will bind to DNA in a test tube, leading to destabilization of the normal DNA structure. This destabilization can be reversed by removing the aluminum, allowing the DNA to return to its normal configuration. Neurons with neurofibrillary degeneration (due to aluminum injections in animals or to Alzheimer's disease in man) are functionally impaired but do not rapidly deteriorate and die. This derangement of the many nerve cells with neurofibrillary degeneration may contribute to the severe mental impairments seen in patients with Alzheimer's disease.

In our work investigating the significance of this relationship between aluminum accumulations and Alzheimer's disease, we have measured serum aluminum concentrations in patients with Alzheimer's disease and compared those measurements with concentrations found in other hospitalized patients of similar age and gender. The average serum aluminum concentrations were similar to those of elderly patients without the disease. Several young and elderly normal volunteers, also, were found to have serum aluminum concen trations in the same range. No significant correlations were found between age, race or gender and serum aluminum concentration in the cerebrospinal fluid of several groups of patients and there was no significant differences among groups. Cerebrospinal fluid aluminum concentrations were similar in patients with Alzheimer's disease, those with other brain diseases and chronic schizophrenic patients matched for age. The results tend to confirm that the increases in nerve cell nucleus aluminum reported in Alzheimer's disease are not the result of a generalized overload of this metal in biological fluids.

The accumulation of aluminum in the brain has been implicated, also, as a factor in the development of "dialysis dementia" found in patients with chronic kidney failure on long-term dialysis treatment. Parathyroid hormone is typically elevated in such patients and this may be related to the aluminum accumulation. To investigate whether parathyroid hormone elevations may cause increased brain aluminum concentrations in patients with Alzheimer's disease, we measured the amount of this hormone in the blood of patients with Alzheimer's disease as well as patients of the same age and gender without the disease ("control patients"). There was no significant difference in average serum pathathyroid hormone concentrations between the Alzheimer's disease patients and the control patients.

If aluminum is a contributor to the degenerative brain changes in Alzheimer's disease, attempts to remove this metal from the body could have significant effects on the course of illness. In this regard, the fluoride ion is of particular interest since elimination of aluminum by urine and feces is significantly increased by fluoride and aluminum retention in the body, reportedly, is decreased. A mutual reaction may occur between these ions in the body, resulting in the formation of an aluminum fluoride complex with the result that aluminum is not retained in the organism.

Consistent with this hypothesis, a recent study found a lower annual incidence of Alzheimer's disease in a count with high fluoride concentration in the water supply. Whether this effect is due to inhibition of aluminum absorption or enhanced excretion of aluminum from the body is unknown. Also, this study must be replicated before its results can be accepted as valid.

We have been doing a series of animal experiments to see in what ways fluoride may pervent or reverse the brain abnormalities produced by aluminum. These projects are being carried out to study directly the effects of aluminum on the brains of animals, effects which cannot be studied in human subjects.

In addition, we are not beginning a study of effects of sodium fluoride on the course and outcome of Alzheimer's disease. Prospective subjects are being evaluated and those patients meeting diagnostic criteria for Alzheimer's disease are being included in the study.

Progression of the disease in patients while on fluoride is being compared with those on an inactive compound (placebo) to discover whether the course of the illness can be altered. Fluoride (10-20 mg per day) will be given for six months and a placebo will be given for six months. Psychological testing is being administered as well. Results are being documented and psychological and laboratory tests repeated periodically to determine course and outcome and to monitor the effects of treatment. We, also, will be able to compare the results of laboratory and psychological tests on patients with Alzheimer's disease with those of healthy relatives to learn more about the disease process.

B. Pilocarpine Therapy Of Organic Dementia

Four separate studies of independent groups of investigators have recently found a significant reduction of choline acetyltransferase, a synthetic enzyme for acetycholine in the brains of patients with Alzheimer's disease, compared with age-and sex-

matched controls. The depletion may be specific and may occur with relative preservation of postsynaptic muscarinic receptors.

The above neurochemical abnormality and the existing body of evidence implicating acetylcholine in memory mechanisms strongly suggest the potential therapeutic application of cholinomimetics in memory disorders such as Alzheimer's disease.

The use of a cholinergic agonist which can bypass the synthetic enzyme is clearly indicated. Unfortunately, the otherwise potent muscarinic agonist oxotremorine is unsafe for human use and arecoline is too short-lived for practical purposes. Pilocarpine, on the other hand, may be particularly suitable since it is safe, can be ingested orally, and has a longer duration of action than arecoline.

Pilocarpine is a natural alkaloid which stimulates directly the same autonomic effect cells as those acted upon by cholinergic postganglionic nerve impluse, and, in addition, has a direct cholinomimetic effect in the central nervous system. When injected into the cerebral ventricles of man or rabbit, a variety of autonomic and somatic motor effects occur which suggest the activation of paraventricular hypothalamic nuclei. When injected intravenously in low doses in cats, pilocarpine evokes a characteristic cortical arousal or activation response, similar to that produced by the injection of anticholinesterase agents, which is blocked by atropine. Recent studies have shown that pilocarpine acts at cholinoceptive sites. Finally, intraventricular pilocarpine in cats produced behavioral, autonomic and motor responses that are blocked by intraventricular scopolamine but not by intraventricular ganglionic or neuromuscular blocking agents.

A double-blind study is being conducted to compare the effect of oral pilocarpine with placebo on a number of cognitive and behavioral measures. Memory tests include both short- and long-term recall of standardized word lists.

A therapeutic trial of pilocarpine, a cholinergic agonist in patients with organic dementia is administered. The dose of pilocarpine ranges from a starting oral dose of 2.5 mg up to a maximum of 25 mg a day for two (2) weeks. A peripheral anticholinergic agent (Robinul) is being coadministered (1-2 mg three times a day) to reduce pilocarpine side effects.

At this time, data are being collected and analyzed. Preliminary findings indicate that subjects in the experimental group show a greater response than those in the control group.

C. Lecithin And Tetrahydroaminoacridine In Patients With Alzheimer's Disease

Central cholinergic underactivity and overactivity have been hypothesized to play a role in mania and depression respectively. Recently a specific deficiency of choline acetyltransferase, a synthetic enzyme of acetylcholine, was reported in the hippocampus of Alzheimer's disease. A growing body of evidence suggests that the central cholinergic defect may explain the profound disturbance of memory functions in Alzheimer's and Huntington's disease. The use of cholinergic agents in these disorders seems warranted.

Lecithin, the major dietary source of choline, a precursor of acetylcholine, has recently been reported to be much more effective than choline itself in raising brain acetylcholine levels. Tetrahydroaminoacridine is a centrally acting anticholinesterase agent with very minimal peripheral side effects unlike other anticholinesterases such as physostigmine. Tetrahydroaminoacridine, also, is a respiratory stimulant and antagonizes morphine-induced respiratory depression.

The rationale of combining lecithin and tetrahydroaminoacridine would be to enhance both presynaptic accumulation and release of acetycholine (via lecithin) and also increase postsynaptic activity of acetylcholine by the anticholinesterase action of tetrahydroaminoacridine. By this combined approach of using two drugs which by themselves have very low toxicity and side effects, we hope to achieve significant increase in cholinergic activation.

Sleep apnea is a syndrome characterized by repeated apneic episodes of at least 10 seconds duration occurring during sleep (often more than 200-400 episodes on a single night). This is accompained by snoring, disturbed sleep and daytime hypersomnolence. The reason for the periodic failure of the respiratory mechanism is unclear. No medical treatment exists to correct the respiratory disorder except for one reported case of adult apnea treated with tegretol and another case of sudden infant death syndrome treated with theophylline, a respiratory stimulant. Only tetrahydroaminoacridine is being used in the sleep apnea patients.

In the neuropsychiatric patients, only lecithin is administered for the first five (5) weeks. In the second five (5) week period, both lecithin and tetrahydroaminoacridine are administered. Mood and behavior ratings and memory testing are performed twice weekly and sleep EEG monitoring occurs every two (2) to three (3) nights. Blood samples are drawn to measure choline levels.

In the sleep apnea patients, the first week's administration of tetrahydroaminoacridine is oral. In the second week one (1) intraveneous infusion is administered. Subjects' EEG and EKG patterns are monitored. Respiration is monitored with nasal thermisers and abdominal and chest strain gauges. The number of apneic episodes are counted for each night.

Data are being collected at this time. Preliminary findings show, however, a greater response in the subjects than in the controls.

D. Regional Cerebral Blood Flow

In 1965, a non-invasive method for monitoring regional cerebral blood flow was introduced. This method involves inhalation of ^{133}xenon, a radioisotope of xenon gas. Since a primary determinant of regional cerebral blood flow is regional cerebral metabolic activity, the potential of this method for studying cerebral activity in living patients is apparent. This method has been refined and applied to the clinical study of cerebral activity in healthy individuals and in various disease states.

An abnormal distribution of cerebral activity in schizophrenic patients has been found in both states of rest and cognitive testing. Specifically, hypofrontal and hyperparietal flows have been observed, the inverse of the normal distribution.

Furthermore, the schizophrenic patients failed to activate their frontal lobes during cognitive testing. The only other group of psychiatric patients studied have been patients with dementia. From the results of this work it appears possible that the distribution of activity in patients with Alzheimer's disease may be diagnostic.

The observations made in psychotic and demented patients may provide further clues about the pathophysiology and neuropsychology of these disorders. We propose to attempt to replicate the work already described and, further, to explore the potential of ^{133} xenon inhalation in regional cerebral blood flow as a clinical and reseach tool in neuropsychiatry.

Subjects are being drawn from several sources to address such issues as the effects of age, gender, different psychiatric diagnoses, and different treatments. Normal, healthy, non-pregnant volunteers of similar age are being recruited to provide control values.

Patients between the ages of 20 and 80, who have either a psychotic diagnosis, organic brain syndrome or dementia are being studied. We are performing up to six inhalation procedures on a given patient during a one year period, and up to three procedures in a year on normal volunteers. This allows for repeat testing under different treatment conditions, e.g., while off neuroleptic drug versus while receiving neuroleptic drugs.

Several activation conditions will be explored in addition to the normal resting condition. Each subject is undergoing three inhalations per session. Subjects are performing cognitive tasks such as Miller Analogies, continuous performance tasks, or other attention and spacial tasks during the eleven minute regional cerebral blood flow procedure. These tasks are being administered to assist us in our evaluation of possible disorders of cerebral activation.

IV. Parkinson's Disease

A. Brain Grafts

Many neurological disorders are associated with degeneration of discrete populations of neuronal elements. Parkinson's disease, for example, manifested primarily by abnormalities of movement and posture, is characterized by neuronal loss and gliosis in the dopamine-containing zona compata of the substantia nigra. Neurochemically, the syndrome is characterized by decreased concentrations of dopamine and its principal metabolite, homovanillic acid in the striatum, and substantia nigra.

Current therapeutic approaches to Parkinsons's disease involve peripheral administration of L-DOPA, a precursor for dopamine, and dopamine-like agents. In practice, despite some dramatic improvements, such therapeutic regimens are frequently not completely effective, or associated with severe untoward side effects. Many of these difficulties may result from: (i) use of a precursor rather than the transmitter substance itself; (ii) generalized distribution of the drugs in brain than focal release from terminals in discrete areas; (iii) absence of the phychological mechanisms which normally regulate transmitter release from dopaminergic terminals.

We have attempted to circumvent these problems by using central nervous system grafts as a source of dopamine in an animal model of substantia nigra degeneration. We have found that fetal rat substantia nigra neurons can be homologously transplanted in juxtaposition to dopamine-denervated host adult caudate. The grafts proliferate extensively in situ and establish a behaviorally appropriate functional input to the denervated caudate.

As an experimental model, we chose the rat with unilateral destruction of the substantia nigra. Within one week following an unilateral injection of 6-hydroxydopamine (a neurotoxin) into the substantia nigra, dopamine-containing cells in that site, and their axonal terminals in the corpus striatum degenerate; dopamine concentrations in the corpus striatum are greatly reduced. To compensate for the loss of dopamine, it is postulated that striatal neurons develop increased sensitivity to dopamine. This "supersensitivity" is manifested as: increased numbers of dopamine receptor sites and increased dopamine-stimulated adenylate cyclase activity, a decrease in the threshold of caudate neurons to the inhibitory effects of iontophoretically applied dopamine agonists, and a pronounced rotation contralateral to the lesion, after systemic administration of the dopamine receptor agonist, apomorphine. If a brain graft developed a dopamine input to the denervated caudate nucleus, we postulated that the supersensitivity, manifested as apomorphine-induced rotation, would be reduced.

Unilateral destruction of dopamine cell bodies in male Sprague-Dawley rats was accomplished using injection of 6-hydroxydopamine into the right substantia nigra and ascending dopamine axon bundle. After 2-4 months, the rats were placed in a rotometer and given a subcutaneous injection of apomorphine hydrochloride. The number of clockwise and counterclockwise turns was recorded separately for each animal at 5-minute intervals for 40 minutes. Testing was repeated twice a week for 3-4 weeks.

Rats that had stable patterns of rotation were subjected to grafting operations. Pieces of fetal ventral mesencephalon containing the substantia nigra or adult sciatic nerve were dissected out and injected into the lateral cerebral ventricle ipsilateral to the 6-hydroxydopamine lesion. Baseline turning rates were not statistically different for the animals in substantia nigra and sciatic nerve implant groups prior to transplantation.

Four weeks after grafting, animals were again tested for rotation in response to the same dose of apomorphine (twice a week for 3-4 weeks). By and large, most animals did not rotate as much after grafting as before. The reduction in turning was significantly greater for animals grafted with substantia nigra tissue than for animals with sciatic nerve implants. The rotatory response reduction for the substantia nigra transplant in rats was 60 percent (see Figure 2).

Although the sciatic nerve fibers degenerated after grafting, the histochemical studies revealed excellent survival and growth of the ventral mesencephalic substantia nigra grafts. Of 30 monoamine-containing implants examined in nine animals, all but one showed large numbers of catecholamine and serotonin containing neurons with a morphology typical of adult substantia nigra and raphe, respectively. The catecholamine and serotonin containing cell bodies formed one or more dense clusters in the

graft which were clearly separate. Monoamine containing fibers proliferated extensively within the grafts and invaded the adjacent caudate in all nine animals with substantia nigra implants. The catecholamine fibers that grew into the denervated caudate formed a network only in the part of the caudate adjacent to the graft. A similar ingrowth was seen into the lateral septum. No monoamine containing fibers, however, invaded the corpus callosu, even at points of attachment of the graft. Monoamine containing fibers also formed dense sheets of fibers within the ependyma of the lateral ventricle. For the most part, anteriorly placed grafts formed well-demarcated structures in the lateral ventricle opposed to or fused with the medial surface of the caudate and lateral surface of the septum. Some deeper grafts lay interstitially, fused with the host brain tissue. Transplants that were placed more posteriorly in the lateral ventricle were usually found adjacent to or fused with the fornix and dorsal alveus of the hippocampus. There was little or no evidence of any scaring or pathological disturbances of the host striatum.

It was of great interest to compare the histochemical properties of grafts in animals that showed reduced turning after grafting with those that did not. Both groups had approximately equal mean numbers of catecholamine-containing cell bodies per animal and proliferating fibers in the graft. Moreover, the ingrowth of fibers into the adjacent caudate was approximately equal in both groups.

Although numerous studies have demonstrated (i) the regeneration of mammalian nervous tissue following injury, (ii) that various parts of the mammalian central nervous system can be grafted to peripheral tissue, and (iii) that non-nervous tissue can be grafted to the brain, there have been few studies demonstrating that central nervous system tissue could be grafted to and survive in the brain or spinal cord of another animal. In some studies where the grafted nervous tissue has been placed into host brain areas, fiber proliferation and synpase formation have been demonstrated. This is, to our knowledge, the first demonstration, however, that the grafting of mammalian brain tissue from one animal to another elicits an alteration in the behavior of the recipient animal consistent with the normal function of the grafted tissue.

The progression of this work has been to introduce rhesus monkeys into the project. Substantia nigra or adrenal medulla grafts have been implanted in six rhesus monkeys. Some of these grafts have survived. These have typical catecholamine-containing cells and fibers, some of which penetrated into the caudate nucleus. In addition, an electrophysiological study of grafted substania nigra cells has shown that these cells are spontaneously active.

Work is in progress involving the implentation of grafts of minced fetal cortex or telencephalon into neonatal animals. These grafts are found to survive as large structures, fused and well integrated with the host cortex. It is hoped that this work might provide information that could make it possible to partially reverse the anatomical and behavioral consequences of early brain damage in rats using these techniques.

When the direction of these graft studies are considered in light of possible, future applicability to humans, advancement in the treatment of Parkinson's disease is considered initially. Parkinson's disease has a wide variety of signs and symptoms; not

all of these are reliably improved by current therapy. Our data show that catecholamine-containing tissue, implanted in proximity to the caudate survives, proliferates and can produce improvements in lesion-induced motor abnormalities. While clearly much additional research is required to establish the safety and long term stability of grafts, there is a possibility that such a local catecholamine source could offer the patient with Parkinson disease a more physiological treatment than is presently available. In this context, it is important to explore autografts and/or homografts of peripheral catecholamine-containing tissues, such as adrenal medulla. If further studies can establish the efficacy and safety of this approach for therapy, it may eventually be possible to employ techniques similar to those that we have used here in the treatment of other neurological disorders where the loss of nerve cells is reasonably well circumscribed.

B. Chronic Deprenyl Treatment To Parkinsonians

Deprenyl, a selective inhibitor of monoamine oxidase Type B has been reported to supplement the beneficial effects of L-DOPA in the treatment of patients with parkinsonism. In view of the fact that chronic deprenyl intake elevates the brain concentration of phenylethylamine and also gives rise to both amphetamine and methamphetamine in brain and urine, there is a need for more information on the in vivo mode of action of this drug. It is not clear if the behavioral responses to chronic deprenyl treatment are related to production of amphetamine and methamphetamine or to inhibition of monoamine oxidase or some combination of these.

We have studied the therapeutic effects of chronic deprenyl treatment on parkinsonian patients who were simultaneously treated with Sinemet (L-DOPA plus carbidopa) and analyzed the urine for excretion of phenylethylamine and other related biogenic amines and their metabolities. We have found biochemical evidence that directly implicates amphetamine and methamphetamine in the euphoric and other therapeutic effects of deprenyl in parkinsonian patients.

Urines were collected from a group of five parkinsonian patients who were on Sinemet before and after taking 10 mg (-) deprenyl per 24 hours for periods ranging from 12 days to 2 months. The volumes were measured and aliquots separated and stored at -40°C until analyzed. Urines were also collected from a group of six depressed patients before and after taking 10 mg (-) deprenyl per day for over four weeks. Biochemical determinations were performed "blindly".

Amphetamine and methamphetamine were detected in measureable quantities in the urine of the parkinsonian (Table 1) and depressed patients (Table 2) after chronic deprenyl treamtents. The excretion of methamphetamine ranged between one and seven mg/24 hours. The ratio of metamphetamine to amphetamine in these urines ranged for one to three. We also analyzed urines from two parkinsonian patients eight and 15 days after terminating deprenyl treatment. Both of these deprenyl metabolites were detected in measureable amounts, thus indicating either that they and/or deprenyl or both are slowly eliminated from the body.

Chronic treatment of the parkinsonian patients (who were on Sinemet throughout the period of study) with deprenyl significantly increased the urinary output of phenylethylamine and p-tyramine, with phenylethylamine (Table 3) showing the great-

est change. The increase in phenylethylamine was not as great, however, as that found in the depressed patients treated with deprenyl (Table 2). This difference is very likely due to the intake of carbidopa in the Sinemet; a drug that inhibits peripheral decarboxylation of a variety of amino acids including phenylalanine. Deprenyl treatment of the parkinsonian patients, on the other hand, failed to decrease the excretion of the major metabolites of phenylethylamine (phenylacetic acid) and p-tyramine (p-hydroxyphenylacetic acid) (Table 3).

In conclusion, our research indicates that chronic treatment of parkinsonian patients with deprenyl increased urinary output of phenylethylamine, n-and p-tyramine without significantly changing the excretion of their metabolites. These effects apparently are directly related to inhibition of monoamine oxidase Type B in the central nervous system. Deprenyl is metabolized to amphetamine and methamphetamine, metabolites which may be responsible for some of the biochemical changes in the excretion and metabolism of the various biogenic amines measured and as such may play a role in the therapeutic action of deprenyl in parkinsonism.

Table 1

Urine Excretion of Amphetamine and Methamphetamine in Parkinsonians after Chronic Deprenyl (10 mg/24) Treatment*

Patient No.	Amphetamine ug/24 hr	Methamphetamine ug/24 hr	Methamphetamine-Amphetamine ratio
1	245	381	1.6
2	723	1624	2.7
3	336	623	1.9
4	284	893	3.1
5	999	1983	2.0
Mean ± S.E.M.	517 ± 147	1100 ± 303	2.3 ± 0.3

* See Table 1 for information on gender, age, and duration of deprenyl treatment

Table 2

Urine Excretion of Amphetamine, Methamphetamine and Phenylethylamine (PEA) in Depressed Patients after Chronic (-) Deprenyl (10 mg/24 hr for over 4 weeks)

Patient No.	Amphetamine ug/24 hr	Methamphetamine ug/24 hr	PEA (Baseline) ug/24 hr	PEA after chronic deprenyl ug/24 hr
		Chronic Deprenyl		
1	2996	6799	2.6	409
2	791	1781	1.5	534
3	791	2353	9.9	191
4	2348	4771	1.3	302
5	1137	2568	3.4	106
6	2334	5732	5.3	293
Mean ± S.E.M.	1732 ± 385	4000 ± 838	4.0 ± 1.3	306 ± 62

Table 3

The Effects (mean ± S.E.M.) of Chronic Deprenyl Treatment[a] in Patients with Parkinsonism on the Excretion of Non-Catecholic Phenylethylamines and their Metabolites

Description (n)	PEA (ug/24 hr)	Phenylacetic Acid (mg/24 hr)	P-Tyramine (mg/24 hr)	P-Hydroxyphenyl-acetic Acid (mg/24 hr)	M-Tyramine (mg/24 hr)
Baseline (5)	3.4 ± 1.0	187 ± 43	260 ± 24	4385 ± 716	62 ± 16
Deprenyl (5)	12.7 ± 2.8**	149 ± 17	515 ± 120*	4346 ± 804	118 ± 17*
Percent Charge	37%	-20%	198%	-1%	190%

[a]Deprenyl (10 mg/kg) was given for periods ranging between 12 days and 2 months (see Table 1). *$p < 0.01$ by paired two-tailed t-test; **$p < 0.005$ by paired two-tailed t-test

Table 4

The Effects (mean ± S.E.M) of Chronic Deprenyl Treatment[a] in Parkinsonian Patients on the Excretion (mg/24 hr) of Metabolites Derived from P-Octopamine and Catecholamines

Description (n)	P-Hydroxymandelic acid	VMA	MHPG	DOPAC	HVA
Baseline (5)	1.8 ± 0.1	1.6 ± 0.6	2.5 ± 0.2	5.3 ± 0.8	7.6 ± 1.4
Deprenyl (5)	1.7 ± 0.3	0.7 ± 0.1*	1.9 ± 0.1*	3.4 ± 0.8	7.2 ± 0.8
Percent Change	-5%	-56%	-24%	-36%	-5%

[a]Deprenyl (10 mg/day) was given for periods ranging between 12 days and 2 months (see Table 1).

*$p < 0.05$ by paired two-tailed test

VII. Sleep Studies

A. Sleep Studies of Patients with Cognitive Disorders

A number of studies have demonstrated alterations in the waking and sleeping EEG records of at least some dementia patients and some age controls. In general, these studies describe a tendency toward slowing and flattening of the waking EEG in both normal aging and in dementia. There is a reduction in and slowing of alpha rhythm and an increased prominence of delta and theta activity. In some dementia patients, a correlation between cognitive deficits and EEG abnormalities has been demonstrated.

The sleep records of aged patients are said to show an increase in the number of awakenings and total time awake thoughout the night. Sleep latency, also, is prolonged. Stage 4 sleep is said to be markedly reduced, as is total REM (rapid eye movement) time. The basic REM - Non-REM cycle, however, it said to remain intact. Research further indicates that patients with senile dementias have a larger decrease in Stage 4 sleep and REM time than aged normals without significant evidence of cognitive deficit. These data are, however, controversial.

To attempt to illuminate the issues involved, our study examines the basic sleep cycle in normal aging and in patients with senile and pre-senile dementia. In addition, comparison of sleep data in patients with senile and pre-senile dementia will be obtained as compared with the prior studies which only looked at senile dementia.

Subjects are to be monitored in blocks of three to five consecutive nights at regularly scheduled intervals. Baseline monitoring will be followed by studies of sleep

as drug trials begin, during the drug trials and during and after withdrawal. If the data warrants more intensive investigation, some subjects may be asked to participate for up to 30 consecutive nights.

B. Sleep and Growth Hormone Secretion

It has been known since the late 1960's that growth hormone is secreted in one large episode occurring about 90 minutes after sleep onset in human beings. This one episode accounts for up to 80% of the 24 hour secretion of the hormone. It is not known why this close association of growth hormone secretion to sleep exists. One hypothesis is that sleep is a permissive state for growth hormone to induce growth. Stating the hypothesis more explicitly, only during sleep can growth hormone enhance protein synthesis and related effects.

We hope that to test this hypothesis by using a standard model for the rate of growth of tissues which is the amount of ornithine decarboxylase activity. This is measured in an assay in which Carbon 14, labelled ornithine, is exposed to hepatic microsome preparations and the amount of radioactive CO_2 given off by the reaction mixture is ascertained. In this study rats will be divided into two groups, those that are allowed to sleep and those kept awake. If sleep is indeed a permissive state for growth hormone to induce growth, it seems possible that after growth hormone is injected into rats who are allowed to sleep there will be a greater increase in ornithine decarboxylase activity compared to activity in animals kept awake. We are currently exploring this possibly.

Publications

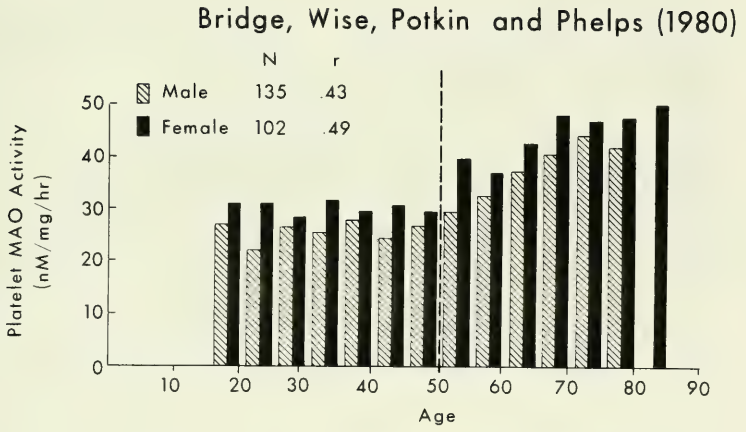
Shore, D., Millson, M., Holtz, J., King, S., Bridge, T. P., Wagner, R. L., DeLisi, L., and Wyatt, R. J.: Serum aluminum in primary senile dementia. Biological Psychiatry 15:971-977, 1980.

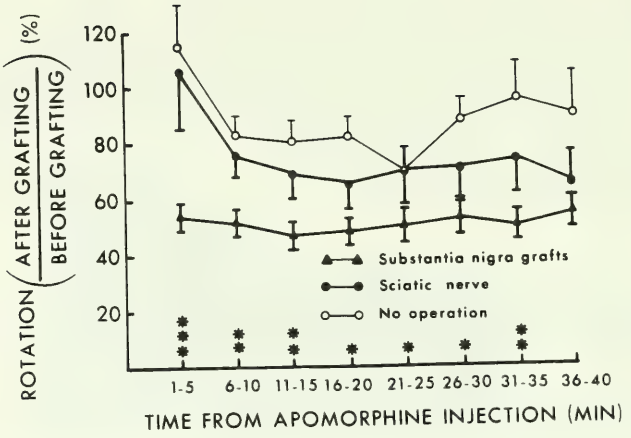
Gillin, J. C., Duncan, W. C., Murphy, D. L., Post, R. M., Wehr, T. A., Goodwin, F. K., Wyatt, R. J., and Bunney, W. E., Jr.: Age-related changes in sleep in depressed and normal subjects. Psychiatry Research 4:73-78, 1981.

Freed, W.J. and Wyatt, R.J.: Transplantation of eyes to the adult rat brain: Histological findings and light-evoked potential response. Life Sciences 27: 503-510, 1981.

Freed, W.J., Perlow, M.J., Karoum, F., Seiger, A., Olson, L., Hoffer, B. and Wyatt, R.J.: Restoration of dopaminergic function by grafting of fetal rat substantia nigra to the caudate nucleus: Long-term behavioral biochemical and histochemical studies. Annals of Neurology, 8: 510-519.

Shore, D., King, S., Kaye, W., Torrey, E. F., Winfrey, H. J., Potkin, S. G., Weinberger, D. R., Savory, J., Wills, M. R., and Wyatt, R. J.: Serum and cerebrospinal fluid aluminum and circulating parathyroid hormone in primary degenerative (senile) dementia. Neurotoxicology (in press).


Bridge, Wise, Potkin and Phelps (1980)
N r
Male 135 .43
Female 102 .49
Platelet MAO Activity (nM/mg/hr)
0
10
20
30
40
50
10 20 30 40 50 60 70 80 90
Age


ROTATION (AFTER GRAFTING / BEFORE GRAFTING) (%)
120
100
80
60
40
20
Substantia nigra grafts
Sciatic nerve
No operation
1-5
6-10
11-15
16-20
21-25
26-30
31-35
36-40
TIME FROM APOMORPHINE INJECTION (MIN)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01500-09 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Indolealkylamines and neuronal function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Economou	Guest Worker	SMRP	NIMH
	Z. Lackovic	Visiting Associate	SMRP	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our objective is to determine the role of serotonin in spinal cord and whether serotonin plays a role in peripheral nerve function.

Project Description:

Our objective is to determine whether serotonin might play a role in the processing of information in the peripheral nervous system and to investigate its role in spinal cord function.

We developed a simple high pressure liquid chromatographic method for 5-hydroxyindoles that will be used for the analysis of samples. Toxins, such as 5,7-dihydroxytryptamine which is relatively specific for serotonin-containing nerves, will be administered to animals. Specific brain and spinal cord lesions will also be performed. Our initial studies reveal an uneven distribution of serotonin and metabolites in spinal cord as well as in the spinal nerve roots. There is also nonuniform distribution of serotonin in blood vessels and peripheral organs. These preliminary studies provide suggestive evidence that serotonin may be a neurotransmitter in the peripheral nervous system. They also suggest that some peripheral serotonin-containing nerves may originate from spinal cord. Our studies are designed to provide a better understanding of the role of serotonin as a neurotransmitter and how drugs might interact with serotonin neurons.

In the future we will expand our initial studies and include a search for peripheral serotonin receptor sites.

Publication:

Lackovic, Z., Parenti, M., and Neff, N.H.: Simultaneous determination of femtomole quantities of 5-hydroxytryptophan, serotonin and 5-hydroxyindoleacetic acid in brain using HPLC with electrochemical detection. Eur. J. Pharmacol. 69: 347-352, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01503-07 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Pharmacology studies of acetylcholine turnover: Control of cholinergic pathways

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. L. Cheney	Chief	SMRP-M	NIMH
Other:	N. Brunello	Visiting Associate	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pentobarbital-induced narcosis may be reversed by intraseptal injection of bicuculline, thyroid-releasing hormone and kainic acid. Moreover pentobarbital produces a dose dependent decrease in the turnover rate of acetylcholine in cortex and hippocampus but not in striatum. The decrease in hippocampus may be reversed by slow infusion into septum of bicuculline, thyroid releasing hormone and kainic acid. The data suggest that the antagonism of the pentobarbital induced decrease in acetylcholine turnover and the narcosis may be modulated through an action on the gamma-aminobutyric acid interneurons in the lateral septum.

Project Description:

The objective of this study was to improve our knowledge of the local circuitry regulating the cholinergic pathway.

To study turnover dynamics rats were infused with phosphoryl($^{3}H_{9}$)choline and the tissue content of choline, acetylcholine and their deuterated analogs was determined using gas chromatography-mass fragmentography. From the percent incorporation of deuterium in acetylcholine and choline the fractional rate constant for acetylcholine efflux was calculated. The turnover rate of acetylcholine was obtained by multiplying the fractional rate constant by the steady-state content of acetylcholine.

The narcosis associated with the administration of pentobarbital may be reversed by intraseptal injection of such chemically diverse compounds as bicuculline, a potent gamma-aminobutyric acid antagonist; thyroid releasing hormone, a neuroactive tripeptide; and kainic acid, a rigid analogue of glutamate. Pentobarbital produces a dose dependent decrease in the turnover rate of acetylcholine in cortex and hippocampus but not in striatum. The effect appears to be maximum at 30 min and returns to normal within 15 min of recovering the righting reflex. Slow local infusion of either bicuculline or thyroid releasing hormone into the septum reverses the pentobarbital-induced narcosis and antagonizes the pentobarbital-induced decrease in the hippocampal turnover rate of acetylcholine. Administration of kainic acid into the lateral, but not the medial septum, reduces specifically the glutamic acid decarboxylase activity in the ipsilateral septum without altering the choline acetyltransferase activity or the turnover rate of acetylcholine in the hippocampus. Moreover, kainic acid injected into the lateral septum antagonizes the pentobarbital narcosis and reverses the pentobarbital-induced decrease in the acetylcholine turnover rate in the ipsilateral hippocampus but not in the contralateral hippocampus. It appears that all three compounds antagonize the pentobarbital-induced decrease in hippocampal acetylcholine turnover rate and the pentobarbital narcosis by modulating neurons in the lateral septum, presumably through an action on the gamma-butyric acid interneurons.

To further characterize the neurochemical substrate underlying this antagonism of pentobarbital narcosis, it would be interesting to measure GABA turnover in the septum of pentobarbital-treated animals and to check whether each compound is acting through the same GABAergic interneurons.

Publication:

Costa, E., and Cheney, D.L.: Functional interactions of neurotransmitter systems. In Muller, E.E. (Ed.): Neuroactive Drugs in Endocrinology. Elsevier/North-Holland, 1980, pp. 137-148.

Robinson, S.E., Cheney, D.L., and Costa, E.: Regulation of septal-hippocampal cholinergic neurons by catecholamines. In Pepeu, G., and Ladinsky, H. (Eds): Cholinergic Mechanisms Phylogenetic Aspects, Central and Peripheral Synapses, and Clinical Significance. New York, Plenum Press, 1981, pp. 705-713.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01505-08 SMRP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Acetylcholine and γ-aminobutyric acid turnover rates: Anesthetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. L. Cheney	Chief	SMRP-M	NIMH
Other:	A. Revuelta	Visiting Associate	SMRP	NIMH

COOPERATING UNITS (if any)

Dr. Edward Brunner, Dept. of Anesthesiology, Northwestern University Chicago, Illinois

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Molecular Pharmacodynamics

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The gaseous anesthetics halothane and enflurane were found to reduce the incorporation of ^{13}C from infused ^{13}C-glucose into both glutamate and GABA in all areas examined. However the turnover rate of GABA and the concentrations of glutamate and GABA were unaffected. Pentobarbital, in anesthetic doses, also reduced the incorporation of ^{13}C into both glutamate and GABA, but in this case the turnover rate of GABA was significantly reduced in accumbens, caudate, substantia nigra and septum. The anticonvulsants, diphenylhydantoin and diazepam, have different profiles of activity on GABA turnover rates. Diphenylhydantoin is selective for the accumbens and diazepam reduces the GABA turnover rate in accumbens, caudate and septum.

PHS-6040
(Rev. 10-76)

Project Description:

The objective of these studies was to study the mechanisms that regulate the function of gamma-aminobutyric acid (GABA) containing neurons in the central nervous system following administration of anesthetics and anticonvulsants.

To measure the turnover rate of GABA ^{13}C-glucose was infused and the percent incorporation of ^{13}C into the precursor, glutamate, and the product, GABA, was determined using gas chromatography-mass fragmentography. The fractional rate constant for GABA efflux was calculated from the ratio between the percent of the label incorporated into glutamate and the percent incorporated into GABA. The turnover rate of GABA was obtained by multiplying the fractional rate constant by the steady-state concentration of GABA. Gaseous halothane (1%; 40 min), enflurane (3%; 40 min), pentobarbital (35 mg/kg, i.p.; 30 min), diazepam (3-10 mg/kg; i.p.; 30 min), diphenylhydantoin (10-20 mg/kg; i.p.; 30 min) and Δ^9-tetrahydrocannabinol (0.2-5 mg/kg; i.v.; 30 min) were administered and the rats were killed by focussed microwave irradiation.

Concentrations of glutamate and GABA were similar in the awake state and during anesthesia with halothane and enflurane in all areas examined. Both volatile anesthetics reduced the incorporation of ^{13}C into both glutamate and GABA in all areas. For example, with halothane the reduction was 16% and 26% respectively in the caudate and 33% and 55% respectively in the cortex. With enflurane the reduction was 42% and 40% respectively in the caudate and 52% and 41% in the cortex. GABA turnover rates were not significantly altered in any of the brain areas. Like the gaseous anesthetics pentobarbital did not alter the concentrations of glutamate and GABA in any area examined. Furthermore the incorporation of ^{13}C into both glutamate and GABA was significantly decreased in all areas. However, the decreased incorporation of ^{13}C into GABA was greater than the incorporation of ^{13}C into glutamate in accumbens, caudate, substantia nigra and septum but not in globus pallidus. Thus the turnover rate of GABA was reduced by 60-70% in these four brain areas: accumbens, caudate, substantia nigra and septum.

The anticonvulsant, diphenylhydantoin, is effective in blocking maximal electroshock convulsions but is much less effective in attenuating metrazol induced convulsions. In contrast, diazepam is a potent blocker of metrazol-induced convulsions but relatively weak in maximal electroshock tests. Both compounds reduced the turnover rate of acetylcholine in hippocampus although diazepam had a more general action. These two compounds had different profiles of activity on the turnover rate of GABA in selected brain areas. Diphenylhydantoin selectively reduced the turnover rate of GABA in the accumbens whereas diazepam reduced the turnover rate of GABA in accumbens, caudate and septum. Thus, diphenylhydantoin does not appear to affect the hippocampal turnover rate of acetylcholine through a GABAergic mediated action. Perhaps diazepam is inhibiting the metrazol-induced convulsions and the hippocampus acetylcholine turnover rate through an action via the GABAergic neurons in the septum.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01506-07 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Narcotic analgesics and the regulation of catecholaminergic neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Saiani	Visiting Fellow	SMRP	NIMH
Other:	A. Guidotti	Chief	SMRP-N	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of opiate receptors located on catecholaminergic cells was studied using primary cultures of adrenal chromaffin cells. These cells contain opiate receptors in measurable amounts. Stimulation of these receptors with agonists decreases the release of catecholamines elicited by nicotine. The results suggest that stimulation of opiate receptors modulates allosterically the function of nicotine and other receptors located on the chromaffin cell membranes.

Project Description:

Our objective was to study the action of narcotic analgesics administered acutely or chronically, on the function of various cells that release catecholamines. To establish whether narcotic analgesics interact with catecholaminergic neurons, we studied changes in the release of catecholamines from primary cultures of cow adrenal chromaffin cells.

Results

The primary culture of adrenal chromaffin cells from bovine adrenal medulla is an ideal model to study the molecular mechanisms by which the narcotic analgesics control the function of catecholamine containing cells. We have shown that chromaffin cells in culture contain high affinity, saturable stereospecific binding sites for opiate agonists and antagonists. The binding was characterized with ^{3}H-dihydromorphine, ^{3}H-D-Ala^{2}-D-leu^{5}-enkephalin, ^{3}H-ethylketazocine and ^{3}H-SK&F 10047 to label the μ, δ, κ and σ receptors, respectively. ^{3}H-Etorphine, which labels both μ and δ receptors, was also studied, as well as two antagonists, ^{3}H-naloxone and ^{3}H-diprenorphine. The Kd was similar for all the compounds (approximately 2 nM).

A similar Kd was obtained when we studied the same ^{3}H-ligands in P2 membranes prepared from bovine frontal cortex. In brain, the Bmax for all the compounds ranged from 50 to 80 fmol/mg protein, whereas in adrenal medulla, the Bmax of the various compounds ranged from 20 to 200 fmol/mg protein. ^{3}H-Etorphine, ^{3}H-diprenorphine, ^{3}H-naloxone and ^{3}H-SK&F 10047 bind with a Bmax of 230, 170, 72 and 30 fmol/mg protein, respectively. ^{3}H-Dihydromorphine, ^{3}H-D-Ala^{2}-leu-enkephalin and ^{3}H-ethylketazocine bind with a Bmax less than 20 fmol/mg protein.

In membranes of adrenal medulla, morphine, D-Ala^{2}-D-leu^{5}-enkephalin, beta-endorphin, ethylketazocine and pentazocine displaced ^{3}H-etorphine with a Ki = 100-400 nM, while diprenorphine, phenazocine, FK 33824, naloxone and SK&F 10047 displaced ^{3}H-etorphine with a Ki of 4,12,30,35 and 45 nM, respectively. The potency of morphine and D-Ala^{2}-leu-enkephalin for displacing ^{3}H-etorphine was greater in membranes prepared from frontal cortex than in those prepared from adrenal medulla.

Kinetic studies revealed that frontal cortex possesses two populations of receptors, while the adrenal mdulla has only one, labeled by ^{3}H-etorphine. ^{3}H-Etorphine binding was not displaced by the nonopioid peptides, substance P, bradykinin, oxytocin, neurotensin, and cosyntropin; it was decreased by NaCl (100 mM) and GMp-p (NH)p (50 μM). These results suggested that etorphine receptors in bovine chromaffin cells are different from those in frontal cortex and that they are not of the μ, δ, or κ type. When these receptors are occupied by specific opiate agonists (etorphine, morphine, beta-endorphin, and FK 33824), the number of nicotinic receptors and the amount of catecholamine release induced by nicotine are reduced in a way that reflect the potency of these opiates in displacing the ^{3}H-etorphine in adrenal medulla membranes. The specificity of the action of opiate receptor agonists is supported by the

ability of the opiate receptor antagonists diprenorphine and naloxone to block their effects and by the inability of the opiate receptor agonists to reduce release of catecholamines elicited by K^+ ions.

Conclusions

The opiate receptors are present in membranes of adrenal chromaffin cells. Activation of these receptors causes a non-competitive inhibition of the release of catecholamines elicited by the stimulation of nicotinic receptors. From our data, it can be inferred that when the met-enkephalin-like material in terminals of splanchnic nerve is released, it modulates the release of catecholamines induced by the stimulation of nicotinic receptors elicited by the concomitant neurally mediated release of acetylcholine. Since the Bmax for binding of the antagonist is large, chromaffin cells may contain two types of receptors, one specific for met-enkephalin and the other for beta-endorphin. Beta-endorphin released into the circulation from the intermediate or anterior lobe of the pituitary can influence the function of chromaffin cells.

The interrelations between the opiate agonists and the catecholamine secretion are relevant to the missions of the NIMH in many ways. The present study establish a model to investigate how two neurotransmitters secreted from the same neuron can interact in the regulation of the activity of postsynaptic cells. In our model this activity is represented by the catecholamine secretion.

Publications:

Kumakura, K., Guidotti, A., Yang, H.-Y.T., Saiani, L., and Costa, E.: A role for the opiate peptides that presumably coexist with acetylcholine in splanchnic nerves. In Costa, E., and Trabucchi, M. (Eds.): Neural Peptides and Neuronal Communication. New York, Raven Press, 1980, pp. 571-580.

Costa, E., Guidotti, A., and Saiani, L.: Opiate receptors and adrenal medullary function. Nature 288: 304, 1980.

Costa, E., Guidotti, A., Hanbauer, I., Hexum, T., Saiani, L., Stine, S., and Yang, H.-Y.T.: Regulation of acetylcholine receptors by endogenous cotransmitters: Studies of adrenal medulla. Fed. Proc. 40: 160-165, 1981.

Costa, E., Guidotti, A., Hanbauer, I., Hexum, T., Saiani, L., and Yang, H.-Y.T.: Regulation of cholinergic transmission in adrenal medulla. In Pepeu, G.C. (Ed.): Proceedings of the International Symposium on Cholinergic Mechanisms. Plenum Press, 1981, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01508-11 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Modulation by LSD and adenosine analogs of cholinergic function in various brain areas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Murray	PRAT Fellow	SMRP	NIMH
Other:	D. L. Cheney	Chief	SMRP-M	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

LSD (0.93 μmol/kg i.v.) significantly increased the turnover rate of acetylcholine in the frontal cortex, but not in the striatum or hippocampus of the rat brain. In contrast, an equimolar dose of 2-bromo-LSD, a nonhallucinogenic congener of LSD, did not affect the acetylcholine turnover rate in any of the brain areas examined. Moreover, this effect of LSD on cortical cholinergic dynamics was not mimicked by several putative serotonin agonists. These results argue against a direct postsynaptic serotonin agonist effect of LSD on the soma and/or terminals of cortical cholinergic neurons. The i.vt. administration of 2-chloroadenosine, an adenosine receptor agonist, decreased the turnover rate of acetylcholine in the hippocampus and cortex, but not in the striatum. This effect of 2-chloroadenosine was antagonized by the i.vt. administration of theophylline, an adenosine receptor antagonist, suggesting that an activation of adenosine receptors may be operative in this action of 2-chloroadenosine.

Project Description:

Our objective was to evaluate the effects of LSD and other putative serotonin receptor agonists on cholinergic dynamics in various areas of the rat brain. These studies attempt to achieve a better understanding of the mode of action of LSD and to provide information concerning the possible interactions between serotonergic and cholinergic neurons in the brain. In addition, we have investigated the influence of adenosine analogs and methylxanthines on cholinergic function in the rat brain in an effort to provide information on the role of adenosine as a neuromodulator.

The turnover rate of acetylcholine was determined following an infusion of phosphoryl-$^{2}H_{9}$-choline. The percent of incorporation of deuterium into the precursor, choline, and product, acetylcholine, was determined and the turnover rate was calculated by multiplying the fractional rate constant for acetylcholine efflux by the steady state concentration of this neurotransmitter.

LSD acts as a potent serotonin agonist on the raphe soma to inhibit neuronal firing. An unanswered question is whether LSD acts postsynaptically as a serotonin agonist or antagonist to produce effects in addition to those contingent on the depression of raphe neuronal firing. To this end, we compared the effects of LSD with those of the putative serotonin agonists MK-212, m-chlorophenylpiperazine and quipazine. Twenty minutes after the i.v. administration of LSD (0.93 μmol/kg) the acetylcholine and choline levels were unaltered in all of the brain areas studied. However, this dose of LSD elicited a significant increase in the turnover rate of acetylcholine in the frontal cortex, but not in the hippocampus or striatum. In contrast, an equimolar dose of 2-bromo-LSD, a nonhallucinogenic congener of LSD, did not effect the acetylcholine turnover rate in these three brain areas. Moreover, the effect of LSD on the cortical turnover rate of acetylcholine was not mimicked by any of the putative serotonin agonists when these compounds were administered in doses that produced the "serotonin syndrome". These results argue against a direct postsynaptic serotonin agonist effect of LSD on the soma and/or terminals of cortical cholinergic neurons. However they suggest a potential biochemical marker for the psychotomimetic action of LSD. In an effort to test the hypothesis that the activity of cortical cholinergic neurons could be activated by an LSD induced release of inhibition consequent to the cessation of raphe firing, we examined the effects of depletion of brain serotonin on acetylcholine dynamics. Chronic treatment with para-chlorophenylalanine or intracerebral injection of 5,7-dihydroxytryptamine produced an 80% reduction in forebrain serotonin levels; however, neither treatment influenced the turnover rate of acetylcholine in the frontal cortex. Thus, the role of serotonergic mechanisms in the action of LSD on cortical cholinergic neurons remains to be established. Future experiments will examine the possibility that the effects of LSD on cholinergic dynamics involve a distinct subclass of forebrain serotonin receptors.

The i.vt. administration of 2-chloroadenosine, an adenosine receptor agonist, produces a marked decrease in the turnover rate of acetylcholine in the hippocampus (67% reduction) and cortex (36% reduction), but not in the stria-

tum. This effect of 2-chloroadenosine is antagonized by the i.vt. administration of theophylline, an adenosine receptor antagonist, suggesting that an activation of adenosine receptors is operative in this action of 2-chloroadenosine. Parallel studies using ^{3}H-cyclohexyladenosine to label adenosine A_1 receptors have demonstrated that the highest binding is located in the hippocampus and cerebellum of the rat brain, while the lowest levels of binding sites are in the hypothalamus and spinal cord. Within the hippocampus the density of ^{3}H-cyclohexyladenosine binding sites is highest in area CA_1, while area CA_3, the subiculum, and the dentate gyrus contain equivalent densities approximately 20-25% lower than CA_1. Our results with 2-chloroadenosine provide further support for an neuromodulatory role for adenosine in the central nervous system. Current investigations are evaluating the regulation of adenosine receptors as well as the role of adenosine receptor subtypes in the regulation of cholinergic dynamics.

Publication:

Murray, T.F., and Cheney, D.L.: The effect of phencyclidine on the turnover rate of acetylcholine in various regions of the brain brain. J. Pharmacol. Exp. Ther., in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01509-11 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Pharmacological studies of acetylcholine turnover: effects of diet and stress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Blaker	Staff Fellow	SMRP	NIMH
Other:	N. Brunello	Visiting Associate	SMRP	NIMH
	D. L. Cheney	Chief	SMRP-M	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Increasing choline availability of choline either by adding choline to the diet or by parenteral administration of choline, phosphorylcholine or lecithin only moderately increases brain choline levels without altering brain acetylcholine levels. In hippocampus and cortex but not in striatum, increased choline availability produces a significant reduction in acetylcholine turnover rates. Comparisons in the acetylcholine concentration and turnover rate of eight brain regions in each of seven strains of rats demonstrated interstrain differences as much as 40% in each measure. Large interstrain differences were also found in the acquisition of a conditioned avoidance response. However, no correlation among the strains was found between the response acquisition and either the acetylcholine concentration or turnover rate in any brain structure.

Project Description:

The present studies were concerned with the role of increased availability of choline in the regulation of cholinergic neurons in the CNS and the investigation of possible relationships between learning ability and brain cholinergic function. To measure cholinergic function phosphoryl($^{3}H_{9}$)choline was infused through the tail vein of rats and the incorporation of label into choline and acetylcholine was determined using gas chromatography-mass fragmentography. From the choline and acetylcholine curves representing the change with time of the incorporation of label the fractional rate constant for acetylcholine efflux could be calculated. The fractional rate constant multiplied by the steady-state content of acetylcholine determined the turnover rate of acetylcholine.

Diet: Several studies have indicated that the availability of the precursor of acetylcholine is an important factor regulating the dynamics of acetylcholine metabolism. To clarify this point we have measured the turnover rate of acetylcholine following changes in dietary choline and administration of choline by intraperitoneal injection or intravenous infusion. Rats fed diets supplemented with choline exhibit a 60% increase in blood choline and only a 20-30% increase in striatal or hippocampal choline. When the blood content is further increased by an intraperitoneal injection of choline the hippocampal choline content increases only by 15-20%. Following a rapid rate of infusion of high doses of lecithin, phosphorylcholine or choline for 15 min the blood level increases by 4 or 6 fold but the concentrations of choline in the striatum and cortex are increased by only 0.5, 2 or 3 fold, respectively. Despite the high doses of choline administered with the various procedures the acetylcholine content of various brain areas remains unchanged. The presence of high levels of choline does not produce an increase in the turnover rate of acetylcholine. In fact, in hippocampus and cortex, but not in striatum, there is a significant decrease in the turnover rate of acetylcholine due, perhaps, to a direct receptor activation by choline.

Learning. Naive animals of seven different strains were infused over nine minutes with deuterated phosphorylcholine followed by focused microwave irradiation of the head and the acetylcholine concentration and turnover rate determined in eight brain regions. Although the relative distribution pattern of acetylcholine among the brain regions was similar in all the strains, interstrain differences in the absolute concentration of acetylcholine in particular brain regions were found to be as large as 40%. Similarly large differences in the acetylcholine turnover rate was also noted in some brain regions.

In parallel studies, rats of each strain were tested in a two way shuttlebox apparatus for the acquisition of a conditioned avoidance response. Each rat was given 100 trials (45 minutes) each day for a period of 8-12 days. Although sometimes large animal-to-animal variations within strains were found, it was possible to identify strains having a generally high, low or intermediate performance level. However, no correlation was seen between the conditioned avoidance response performance and either acetylcholine concentration or turnover in any brain region.

Publications:

Costa, E., Tagliamonte, A., Brunello, N., and Cheney, D. L.: Effects of stress on the metabolism of acetylcholine in the cholinergic pathways of extrapyramidal and limbic systems. In Usdin, E., Kvetnansky, R., and Kopin, I. (Eds): Catecholamines and Stress: Recent Advances. Elsevier/North-Holland Publ., 1980, pp. 59-67.

Wood, P. L., Cheney, D. L., and Costa, E.: Interactions of neuropeptides with cholinergic septal hippocampal pathway: Indication for a possible transsynaptic regulation. In Pepeu, G. and Ladinsky, H. (Ed.): Phylogenetic Aspects, Central and Peripheral Synapses and Clinical Significance. Oxford, Plenum Press, 1981, pp. 715-722.

Brunello, N., Tagliamonte, A., Cheney, D. L., and Costa, E.: Effects of immobilization and cold exposure on the turnover rate of acetylcholine in rat brain areas. Neuroscience, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01510-06 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Effects of Δ^9-tetrahydrocannabinol and levonantradol on cholinergic dynamics in rat brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Murray	PRAT Fellow	SMRP	NIMH
Other:	A. V. Revuelta	Visiting Associate	SMRP	NIMH
	D. L. Cheney	Chief	SMRP-M	NIMH

COOPERATING UNITS (if any)
None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Levonantradol is more than 300 times more potent than Δ^9-THC as an analgesic, but only 4.5 times more potent than Δ^9-THC as a cataleptogenic agent. Levonantradol (0.3-3.0 mg/kg, s.c.) decreased the turnover rate of acetylcholine in the hippocampus and striatum. In contrast, the turnover rate of acetylcholine in the parietal cortex was not affected by any dose of levonantradol. This pattern of activity is qualitatively similar to that of psychoactive cannabinoids, but differs from that of narcotic analgesics. Moreover, naltrexone administration did not antagonize the effects of levonantradol on striatal and hippocampal acetylcholine turnover rate. Thus, these results indicate that the pattern of activity on cholinergic dynamics elicited by levonantradol is consistent with a cannabinoid-like rather than an opioid-like mode of action.

Project Description:

The objective of this research project was to determine the effects of Δ^9-THC and levonantradol, a synthetic cannabinoid related compound, on the dynamics of cholinergic neurons in rat brain.

The turnover rate of acetylcholine was determined following an infusion of phosphoryl($^2H_9$)choline. The percent of incorporation of deuterium into precursor, choline, and product, acetylcholine, was calculated. From the interrelationship between the precursor and product, the fractional rate constant for acetylcholine efflux was determined. The turnover rate of acetylcholine was obtained by multiplying the fractional rate constant by the steady state levels of acetylcholine.

Levonantradol is a cannabinoid related analgesic which is 10-30 times more potent than morphine in a variety of animal tests for analgesia. We have investigated levonantradol's analgesic activity using the rat tail flick test. The ED_{50} for levonantradol-induced analgesia was 0.09 mg/kg (s.c.), while Δ^9-THC in doses as high as 30 mg/kg did not produce any significant analgesic effect. Thus, levonantradol is greater than 300 times more potent than Δ^9-THC as an analgesic. In contrast, we have found levonantradol to be only 4.5 times more potent than Δ^9-THC in its ability to produce catalepsy in rats. These results indicate that levonantradol is more potent as an analgesic than as a cataleptogenic agent, while the converse is true for Δ^9-THC.

Levonantradol (0.3-3.0 mg/kg, s.c.) did not alter the acetylcholine or choline content of any of the brain areas examined. However, levonantradol significantly decreased the turnover rate of acetylcholine in the hippocampus and striatum. The extent of the reduction of the turnover rate of acetylcholine is dose dependent with a maximal inhibition of 80% in the hippocampus and 57% in the striatum following the 3 mg/kg dose. In contrast, the turnover rate of acetylcholine in the parietal cortex was not affected by these doses of levonantradol (0.3-3.0 mg/kg). This pattern of activity on cholinergic dynamics elicited by levonantradol is qualitatively similar to that of psychoactive cannabinoids, but differs from that previously reported for narcotic analgesics. Moreover, naltrexone (2 mg/kg, i.p.) administration does not reverse either the effects of levonantradol on striatal and hippocampal acetylcholine turnover, or the levonantradol-induced analgesia.

Thus, our results indicate that the pattern of activity on cholinergic dynamics elicited by levonantradol is consistent with a cannabinoid-like rather than an opiate-like mode of action. Given the postulate that the inhibition of hippocampal cholinergic mechanisms plays a role in the psychoactive effects of Δ^9-THC, our data suggest that levonantradol may produce cannabinoid-like subjective effects in humans.

Publications:

Revuelta, A.V., Cheney, D.L., Costa, E., Lander, N., and Mechoulam, R.: Reduction of hippocampal acetylcholine turnover in rats treated with (-)-delta-

8-tetrahydrocannabinol and its 1',2'-dimethyl-heptyl homolog. Brain Res. 195: 445-452, 1980.

Cheney, D L., Revuelta, A.V., and Costa, E.: Marijuana and cholinergic dynamics. In Pepeu, G., and Ladinsky, H. (Eds.): Cholinergic Mechanisms Phylogenetic Aspects, Central and Peripheral Synapses and Clinical Significance. Oxford, Plenum Press, 1981, pp. 825-832.

Costa, E., Cheney, D L., and Murray, T.F.: Levonantradol-induced inhibition of acetylcholine turnover in rat hippocampus and striatum. J. Clin. Pharmacol., in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01511-08 SMRP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Neuronal monoamines: a study of their integration and regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Guidotti	Chief	SMRP-N	NIMH
Other:	F. Vargas	Visiting Fellow	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Lab. Preclinical Pharmacology

SECTION

Section on Neuroendocrinology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The integrated actions of dopaminergic neurons were investigated in striatum and N. accumbens after long term treatment with antischizophrenic drugs. Long term treatment with classical antischizophrenics produces tolerance to the activation of the DA striatal system. Tolerance is not obServed after long term treatment with atypical antischizophrenics. In striatum, The supersensitivity of DA receptors produced by these drugs is associated with an increase in calcium binding protein (calmodulin). The calmodulin content was determined in striatum and frontal cortex of control subjects and in eleven schizophrenic schizophrenic patients. No differences were observed in the calmodulin content between these two groups.

Project Description:

Although the catecholamine hypothesis of affective disorders has received support during the past decade, today it is evident that catecholamines are not the only transmitter system involved in the pathogenesis of affective disorders. In fact, all aspects of the pathophysiology of various forms of affective disorders cannot be explained by simple alterations of catecholamine metabolism; it is clear, that many other concomitant biochemical physiological, and psychological factors must be involved.

During the past years, many studies have demonstrated the complex anatomical, physiological and biochemical interactions among monoaminergic and other neuronal systems. In this project, we studied the consequences of short- and long term treatment with neuroleptics endowed with antischizophrenic activity on the dopaminergic neurons and on the postsynaptic striatal events that follow the stimulation of DA receptors.

A. Consequences of short and long term treatment with neuroleptics on DA neurons

1. Antischizophrenic drugs that elicit a high incidence of extrapyramidal side effects, termed "classical," preferentially increase dopamine turnover in striatum. The antischizophrenics that cause a low incidence of extrapyramidal side effects, termed "atypical," preferentially increase the turnover of DA in N. accumbens.

2. Long-term treatment with classical (cataleptogenic) and atypical (noncataleptogenic) neuroleptics produces differential effects on TH activity of locus coeruleus, C. striatum and N. accumbens. The activity of TH (Vmax) in locus coeruleus but not in striatum or nucleus accumbens is increased in rat treated for a long period (10 to 90 days) with 0.5 mg/kg/day of haloperidol.

3. After chronic haloperidol treatment, the drug is withdrawn for 3,15, or 30 days. Challenging doses of haloperidol or other classical neuroleptics (pimozide, chlorpromazine, methiotepine) fail to increase the affinity of striatal TH for $DMPH_4$. Such tolerance to haloperidol was also present in N. accumbens; however, in this tissue the tolerance disappears seven days after haloperidol withdrawal. After chronic haloperidol treatment there is no cross tolerance with clozapine or sulpiride-induced increase of striatal TH affinity for $DMPH_4$.

4. Tolerance to long-term treatment with classical neuroleptics was not prevented by long term treatment with Li++. The rat treated chronically with clozapine and sulpiride showed no tolerance to the effects on TH when given challenging doses of clozapine or haloperidol.

Several reasons account for the differential development of tolerance and supersensitivity to the stimualtion of TH in striatum and N. accumbens: among others, a different synaptic wiring in the two nuclei could play a role. For

instance, in N. accumbens, but not in striatum, the GABAergic neurons are innervated by dopaminergic neurons. We plan to determine the precise role of the striato-nigral pathway and striatal and accumbens interneuronal connections in the regulation of DA neuronal function. In particular, we are eager to study the respective influence of nigra and striatal substance P, GABA, and opiate peptides or opiate agonists and antagonists in the regulation of DA activity. In addition, we plan to study the role of cortico-striatal glutamatergic projections. According to general clinical experience the full therapeutic response to a neuroleptic agent may require several weeks and may last for a considerable time after cessation of therapy. There are reasons to believe that the primary actions of neuroleptics, as observed in short term experiments, is only the first response to induce a series of secondary effects, the nature of which has not been fully clarified. We propose to extend our study to the different neuronal systems associated with the DA system and to long term treatment with classical and atypical antipsychotics.

B. Calmodulin content in corpus striatum and frontal cortex of schizophrenics

Long term treatment with classical neuroleptics in addition to the tolerance to the increase of striatal TH elicited by these drugs, generates supersensitivity to the behavioral effects of apomorphine. The supersensitive response of DA receptors is associated with an increase in the striatal content of Ca^{++} binding protein (calmodulin). Based on this knowledge, we reasoned that if calmodulin was fundamental to the processes involved in dopaminergic neurotransmission, then alteration of calmodulin system could be expected in those abnormalities of dopaminergic neurotransmission associated with the behavior disorders of schizophrenia. Therefore, we undertook the study of the calmodulin content in caudate and cerebral cortex from brain of normal and schizophrenic individuals. We were unable to find significant differences between calmodulin content measured in membranes obtained from striatum or cortex of nine control subjects without a psychiatric history and from seven schizophrenic individuals.

Since the beneficial action of dopamine receptor blockers in schizophrenia suggests that dopaminergic transmission may be altered, this alteration might have been secondary to a change in calmodulin content. However, it is interesting to note that just as there is no increase of calmodulin, there is also no increase of DA sensitive adenylate cyclase in schizophrenic brain. We should conclude that the calmodulin in striatum of schizophrenic patients does not play a role in the control of DA responses. Alternatively, we have to bear in mind that the schizophrenic patients' brain tissue that we alalyzed was subjected to chronic neuroleptic treatment at some time before the patient died. Recently, it was reported that chronic neuroleptic drugs may induce an increase in membrane bound calmodulin concentration; an effect that persists for a long time after cessation of drug treatment. Thus, it is not inconceivable to suppose that calmodulin content in brain of schizophrenic patients was low but it returned to a normal level as a consequence of neuroleptic treatment.

Obviously, it would be desirable to measure calmodulin content in the brain of schizophrenic patients not subjected to drug treatment. To collect striata of schizophrenic patients not subjected to neuroleptic treatment and to measure calmodulin content in this tissue is the goal of our future research.

Publications:

Guidotti, A.: Regulation of tyrosine hydroxylase after chronic treatment with classical and atypical antischizophrenic drugs. In Cattabeni, F. Racagni, R. and Spano, P.F. (Eds.): Advances in Biochemical Psychopharmacology, Vol. 24, New York, Raven Press, 1980, pp. 1-8.

Vargas, F., and Guidotti, A.: Calmodulin in Brain of Schizophrenics Neurochemical Research. 4: 674, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01512-08 SMRP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Transmitter interactions in the regulation of pituitary function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. Guidotti Chief SMRP-N NIMH

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LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of GABA, catecholamine and endorphins in the response of hypothalamus to different psychoactive drugs was studied by monitoring pituitary hormone release. Endogenous opiates stimulate PRL and block FSH release by activation of hypothalamic opiate receptors. These opiate receptors may be located on cells containing prolactin releasing factors since median eminence DA neurons or serotoninergic neurons apparently do not mediate the effects of morphine. Stimulation of hypothalamic or brain GABA receptors with muscimol fails to block morphine or haloperidol-induced PRL release. In contrast stimulation of GABA receptors in anterior pituitary prevents the morphine or haloperidol-induced PRL release. GABA receptors with characteristics similar to those of rats were observed also in human anterior pituitary. This suggects a possible physiological role of pituitary GABA receptors, in the release of PRL.

Project Description:

Hypothalamic neuronal mechanisms transsynaptically regulate behavior and secretion of polypeptide releasing factors (RF's). The RF's, in turn, regulate pituitary function by stimulating adenylate cyclase in the anterior pituitary. In order to define the type of neurotransmitters involved in the release of pituitary hormones, the effects of neuroactive drugs were monitored by the measurement of pituitary hormone release.

1) Effect of opiate receptor agonists and antagonists

Injection of β-endorphin into the basomedial hypothalamus induced an increase of PRL release. Naltrexone was able to block this response. Specificity was further demonstrated since a 10-fold higher dose of γ-endorphin (inactive peptide) was ineffective. When injected into the hypothalamus naltrexone itself produced a suppression of PRL release. After deafferentation morphine still stimulated and naltrexone still inhibited PRL release. In contrast to its activity in vivo, β-endorphin ($2x10^{-8}$ M; $2x10^{-7}$) or naltrexone (10^{-4}M) had no effect on PRL release from the pituitary in vitro. In addition, β -endorphin was unable to alter the inhibitory action of $5x10^{-7}$M apomor -phine on in vitro pituitary halves.

Next, we attempted to determine the events that mediate the PRL release elicied by stimulation of hypothalamic opiate receptors. When the DA tonus was potentiate by giving pargyline (a monoaminoxidase inhibitor), PRL release following submaximal doses of morphine or β-endorphin was not altered. In contrast, when the DA activity was reduced by administration of reserpine or haloperidol, pargyline significantly reduced the increase release of PRL elicited by opiate receptor stimulation. In addition, the action of morphine or β -endorphin but not that of reserpine or haloperidol was blocked by naltrexone.

2) Effect of GABA receptor agonists

The rise in serum prolactin (PRL) concentration after haloperidol (0.05 to 0.5 mg/kg i.v.) or morphine (2 to 16 mg/kg s.c.) in male rats and the PRL increase during estrogen treatment (1 mg/day/15 days) to ovariectomized rats was reduced by systemic injection of the GABA receptor agonist muscimol (1 mg/kg i.v.). However, intraventricular or intrahypothalamic injection of muscimol (100-400 ng) was ineffective in blocking PRL release induced by these compounds.

The concentration of muscimol in hypothalamus after local injection was similar to that obtained following systemic injection of an high doses of muscimol. Thus it appears that stimulation of central GABA receptor was not involved in the regulation of PRL release by i.v. muscimol: After this treatment the muscimol content in the anterior pituitary is high whereas the drug is almost absent in the pituitary of rats receiving muscimol intrahypothalamically (100-400 ng). These considerations has suggested that blockade of PRL release by muscimol is due to a stimulation of GABA receptors located

in the pituitary. Indeed, ^{3}H-muscimol and ^{3}H-GABA receptor recognition sites with characteristics similar to those of brain were present in rats anterior pituitary. In addition, muscimol (10^{-8} to 10^{-6} M) reduced the spontaneous release of PRL from anterior pituitary halves incubated, in vitro. This effect was reversed by the GABA receptor antagonist, bicuculline, but not by the dopamine antagonist, sulpiride. These results provide evidence that GABA receptor stimulation in the anterior pituitary can act to regulate the release of PRL.

From these studies it appears that different neurotransmitter systems are involved in the release of specific anterior pituitary hormones. For example, PRL release is under hypothalamic DA and endorphin control but not under a direct hypothalamic GABA control. GABA might control PRL release acting directly at the pituitary level independently from catecholamines. Very recently, we observed that GABA receptors are present in human anterior pituitary. Further efforts are now required to extend these studies to the numerous hypothalamic peptide neurotransmitter substances (neurotensin, somatostatin, substance P, bombesin) which have been shown to influence hormone release. These observations and the demonstration of large concentrations of GABA in human anterior pituitary suggest a possible physiological role of pituitary GABA receptors in the control of PRL release. In addition, if PRL release can be controlled by stimulation of GABA receptors located on the anterior pituitary, peripheral GABA-mimetic drugs that do not enter the brain may represent a relevant approach to the control of the PRL releasing actions of neuroleptics without altering their central effects.

These studies are relevant to understanding total brain function because the hypothalamic neuronal peptides investigated here are known to be widely distributed throughout the brain. The hormone release neurotransmitter function relationship in the hypothalamus- pituitary axis may be used as a tool to learn the action of different psychoactive drugs on neuropeptide transmission.

Publications:

Grandison, L., and Guidotti, A.: Role of anterior pituitary GABA receptor in the control of prolactin release. In Muller, E. (Ed.): Neuroactive Drugs in Endocrinology, 1980, pp. 213-222.

Grandison, L., and Guidotti, A.: GABA and Benodiazepines binding sites in human anterior pituitary tissuse. Endocrinology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01514-09 SMRP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Trans-synaptic control of protein synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	L. Saiani	Visiting Fellow	SMRP
Other	A. Guidotti	Chief	SMRP-N
	E. Costa	Chief	SMRP

COOPERATING UNITS (if any)

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SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In primary culture of chromaffin cells from cow adrenal medulla TH induction is preceded by an activation of cytosol cAPK and by an increase in nuclear protein phosphorylation. Both the induction of TH and the increase of nuclear phosphorylation require that the assembly of microtubular proteins be functional. Antimicrotubular drugs such as colchicine and vinblastine (10^{-5} M) can block the TH induction elicited by 8-Br-cAMP, when the drugs are added less than 15 hours after 8-Br-cAMP. Since colchicine, added within cAMP also prevents the increase in nuclear phosphorylation, it is possible that the assembly of microtubular proteins might be operative in the intracellular translocation and nuclear uptake of catalytic subunits of cAPK activated by the addition of 8-Br-cAMP. In addition, these data support the view that an increase in nuclear protein phosphorylation is an essential step in the mediation of the acceleration of mRNA synthesis and the subsequent increase in TH synthesis elicited by 8-Br-cAMP.

Project Description:

Our objective was to study the molecular mechanisms whereby transsynaptic stimuli induce new synthesis of specific proteins in chromaffin cells of adrenal medulla. In previous studies we reported that in chromaffin cells of rat adrenal medulla, the sequence of molecular events whereby transsynaptic mechanisms regulate the genetic code expression includes an increase in the cAMP/ cGMP concentration ratio, an activation of cAMP-dependent protein kinase (PK) in cytosol and the translocation of the low molecular weight catalytic subunit of this protein kinase from the cytosol to the subcellular particles. The PK of nuclei is not regulated by cAMP but it increases during the transsynaptic induction of tyrosine-3-monooxygenase (TH) because the cAPK catalytic subunits translocate from cytosol to the nucleus. Thus, the activation and translocation of PK, triggered by the initial increase of cAMP, acts as a long range messenger for the transsynaptic expression of the genetic code.

An injection of nicotinic receptor antagonist or the denervation of adrenal medulla reduces and shortens the cAMP elevation caused by cold exposure and prevents the cAPK activation: it inhibits the nuclear translocation of cAPK catalytic subunits and also blocks the induction of TH. Though these experiments demonstrate a temporal succession between the translocation of the catalytic subunits of cAPK to the nucleus, the increase in nuclear protein phosphorylation and the stimulation of gene activity leading to an increase in TH synthesis, they fail to elucidate: 1) the specific regulatory mechanism involved in the nuclear uptake of catalytic subunits of cAPK; 2) the precise role in nuclear function of the subunits of cAPK that translocate to the nucleus; 3) the mechanisms underlying gene transcription specificity; and 4) the necessity of nuclear transloction of cAPK catalytic subunits in eliciting the transsynaptic induction of TH.

In vivo experiments with rat adrenal medulla are not the most suitable means to study transsynaptic regulation of gene expression at the molecular level because rat adrenal medullae provide only a limited amount of purified nuclear material which does not allow a rigorous monitoring of the fine biochemical events occurring in the nuclear chromatin.

We have then decided to use as an in vitro system primary cultures of cow adrenal medulla cells. These cells were used as a model to evaluate the ability of 8-Br-cyclic AMP (8-Br-cAMP) to induce TH and to study the role of cAPK in this induction. This cell culture maintains a constant level of cyclic nucleotides, catecholamines and related enzyme activities for about four weeks.

Exposure of the cells for 5 hrs to 8-Br-cAMP produces 48 hrs later, a dose related longlasting increase in TH activity; 8-Br-cGMP fails to modify TH. The increase in TH activity caused by 8-Br-cAMP is due to an increase of the Vmax and is preceded by an activation of cytosol cAPK associated with a decrease of the total cytosol cAPK. A sustained increase in nuclear phosphory-

lation begins 8 to 12 hrs after 8-Br-cAMP application. The delayed increase in TH activity induced by 8-Br-cAMP is blocked by actinomycin D, cycloheximide, colchicine and vinblastine. This reduction of TH induction elicited by colchicine and vinblastine (10^{-9}M) is observed only when these inhibitors the microtubular protein polymerization were added 4 to 12 hrs after the addition of 8-Br cAMP which is the inducing stimulus. The addition of colchicine 15 hrs after 8-Br cAMP fails to inhibit TH induction. This blockade of TH induction is associated with an inhibition of the increase in nuclear phosphorylation, but is not associated with an inhibition of protein synthesis. The increase of endogenous cAMP and the induction of TH were also produced by cholera toxin. These results suggest that the increase of TH elicited by 8-Br-cAMP is mediated by the translocation of cAPK subunits from cytosol to the nuclei and that this translocation requires the function of the microtubular network. In future studies, we plan to study how the increase in nuclear phosphorylation regulates the expression of the genetic code during the TH induction.

Since in adrenal medulla, the availability of catecholamines and the adrenergic function are regulated by TH and since adrenergic mechanisms have been implicated in the etiology of affective disorders an understanding of the molecular nature of the regulation of the biosynthesis of catecholamines may contribute to a better understanding of the synaptic defects that may be operative in the etiology of mental diseases. In addition, the translocation of cAPK subunits from the cytosol to the nuclei may operate as a basic mechanism in memory and/or learning.

Publications:

Guidotti, A., Chuang, D.M., Kumakura, K., and Costa, E.: Molecular Biology of Tyrosine Hydroxylase Induction; Second Conference on Monoamine Enzymes (Airlie House). In Usdin, E. (Ed.)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01515-08 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Short and long term biochemical changes in cerebellum after treatment with psychoactive drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. S. Shah	Visiting Associate	SMRP	NIMH
Other:	J. P. Chambon	Guest Worker	SMRP	NIMH
	A. Guidotti	Chief	SMRP-N	NIMH

COOPERATING UNITS (if any)

None

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SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Biochemically, the cerebellum is characterized by a large concentration of cGMP, cAMP, and cGMP-dependent protein kinase. Drug-induced changes in GABA receptor function can be easily monitored by measuring changes in these biochemical parameters. It is suggested that diazepam and muscimol (a modulator and a direct GABA receptor agonist, respectively), modulate cerebellar function by altering the cGMP system. We are now investigating whether diphenylhydantoin has its own receptor on cerebellar structures and which are the short and long term biochemical changes associated with the stimulation of this receptor.

Project Description:

The cerebellum is the brain region where the synaptic interactions among various neurons have been determined with greater detail and precision. The project is designed to relate changes of cAMP, cGMP content, protein kinase activities with the release of transmitters in the cerebellar cortex and deep cerebellar nuclei. Such studies are possible in cerebellum because:

1. The climbing fibers and the mossy fibers are the two major excitatory inputs to the Purkinje cells.

2. The function of Purkinje cells is regulated by inhibitory (GABAergic) interneurons.

3. The Purkinje cells are the sole input from the cerebellum and release GABA on the deep cerebellar neurons.

In previous reports we have shown that pharmacological manipulation of climbing or mossy fibers not only change the firing pattern of Purkinje cells but also increase the cGMP content of the cerebellar cortex. Similarly, manipulation of the cerebellar GABAergic function changes the firing rate of the Purkinje cells and the cerebellar cGMP content. When the concentration of cerebellar GABA is increased or GABA receptors are stimulated, the firing rate of Purkinje cells and the cGMP content of cerebellar cortex are reduced. Conversely, when GABA receptors are blocked, the firing rate of Purkinje cells and the cerebellar cGMP content are increased.

These results suggest that two excitatory transmitters released by the parallel or climbing fibers and GABA released by cerebellar interneurons are independently involved in changing cGMP content on the molecular layer of cerebellum. In addition we have established that rat cerebellum contains a large amount of cGMP dependent protein kinase.

Since diazepam modulates cerebellar cGMP content by regulating GABA receptor excitability, we have decided to test whether another anticonvulsant such as diphenylhydantoin which is chemically related to diazepam, was acting on the cGMP-diazepam-GABA interactions operative in cerebellum. As first approach to this problem we have decided to study the binding characteristics of ^{3}H diphenylhydantoin (DPH) to crude synaptic membranes prepared from rat brain.

The binding of ^{3}H-DPH was measured either in fresh P-2 brain synaptosomal membranes or in frozen-thawed and repeatedly washed membrane preparations. Using 50 mM Tris-HCl, 100 mM NaCl, pH 7.4, ^{3}H-DPH binding was determined by filtration technique using GF/B filters in presence and absence of 2.10^{-5}M cold DPH. Both high and low affinity saturable binding sites were observed. The high affinity binding site has a K_D of approximately 10 nM and a Bmax of 100 fmol/mg prot, while the low affinity site has apparent K_D of 500 nM and a Bmax 2-4 pmol/mg prot. The specific binding was not increased by 2 mM Ca^{++}, 2 mM EGTA or 0.05% Triton X-100 treatment while a preincubation of membranes at 37° increased the Bmax of specific binding up to 30%. The Bmax for high affinity

binding sites is highest in cortex and cerebellum. The Bmax value of spinal cord was about 1/3 that of cortex. Binding of ^{3}H-DPH was also found in kidney, heart and lung however the number of sites per unit of protein is smaller than in brain.

Considering that high affinity binding sites for drugs have the potential of revealing the presence of endogenous ligands which modulate neuronal function the studies have the possibility of increasing our understanding of interaction of neuromodulator and primary transmitters. This type of interaction is particularly interesting to neuropharmacology because has been shown to lead to development of neuroactive drugs with low toxicity.

Publications:

Bandle, E., and Guidotti, A.: A simple and rapid method for the assay of cyclic AMP-dependent protein kinase. J. Neurosci. Methods 2: 419-427, 1980.

Shah, D.S., Chambon, J.P., and Guidotti, A.: ^{3}H-Diphenylhydantoin binding to rat brain membrane. Pharmacologist, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01516-08 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biochemical pharmacology of minor tranquilizers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Ebstein	Pharmacologist	SMRP	NIMH
Other:	A. Guidotti	Chief	SMRP-N	NIMH
	D. Konkel	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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Neuroendocrinology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Behavioral studies have shown similarity of action between benzodiazepines (BDZs) and facilitation of GABAergic transmission, indicating that the pharmacological action of BDZs is mediated through GABAergic synapses. The molecular composition of the GABA receptors was studied biochemically. Two separate binding sites (one for ^{3}H-GABA and one for ^{3}H-diazepam) were isolated by differential solubilization from rat brain homogenates with Triton X-100. Another element of the GABA receptor complex has been isolated, an endogenous brain peptide (EBP) that probably represents the endogenous ligand for the BDZ receptor. EBP was prepared from rat brain either by extraction in hot 1N acetic acid or by Triton X-100-treatment of frozen-thawed crude synaptic membranes. Gel chromatography revealed a peak of inhibitory material that is sensitive to proteolytic enzymes. This material inhibits specifically ^{3}H-diazepam binding and resolves into 2 major bands after electrophoresis on a SDS-12% acrylamide gel. Another protein operative in GABA receptor function is GABA modulin, which is being characterized and purified. These data suggest that GABA receptors and BDZ receptors are functionally associated and that an endogenous peptide regulates their function.

Project Description:

Recent evidence from this laboratory has suggested that benzodiazepines, which are the most widely prescribed drugs, may exert a beneficial effect in psychiatric disturbance by a primary action on GABAergic transmission. The present project intends to present new behavioral evidence which favor a mediation of GABA in the anxiolytic action of benzodiazepine and intends to elucidate the molecular mechanisms of action of benzodiazepines (BDZs).

BDZs like muscimol (a direct GABA receptor agonist) and intraventricular nipecotic acid (a blocker of GABA uptake) delayed the onset of convulsions induced by isoniazid in doses that did not protect the animals from strychnine convulsions. Moreover, diazepam and muscimol had a similar potency in preventing the increase of cerebellar cGMP or of pituitary cAMP induced by isoniazid, picrotoxin, harmaline and reserpine. In the nigrostriatal system BDZs in a manner similar to GABA receptor agonists, block the activation of DA neurons induced by antipsychotics.

The increase in punished behavior elicited by benzodiazepines in rats operating in a conflict test situation is potentiated by intraventricular injections of muscimol. Muscimol, injected intraventricularly in doses of 50 to 200 ng also produces a benzodiazepine-like action by increasing the output of punished operating behavior. These data concur with the hypothesis that the pharmacological action of BDZs may be mediated by an effect on GABA neurons.

Studies from our group have suggested that benzodiazepines increase GABAergic transmission by competing with GABA-modulin, on endogenous inhibitor for the Na^+-independent high affinity binding of GABA. It has been reported that benzodiazepines work on specific "anxiety" receptors. One question posed by these results is whether the interaction shown in vitro in brain synaptic membranes between GABA and benzodiazepines could be just the result of homogenization or whether the three entities (GABA recognition site, benzodiazepine recognition site and GABA-modulin) were part of a supramolecular organization constituting the GABA receptor-ionophore complex at the receptive site of the post-junctional neuronal membranes.

In order to elucidate this problem we have decided to initiate studies devoted to the solubilization and partial purification from rat brain cortex homogenates of [^{3}H]γ-aminobutyric acid (GABA) and [^{3}H]diazepam recognition sites and of their endogenous modulators (GABA-modulin and an endogenous compound which displaces specifically bound [^{3}H]diazepam).

A high percentage of GABA binding sites (virtually free of benzodiazepine binding sites) was solubilized from homogenates of rat brain cortex incubated at 0°C with 1% Triton X-100 and a mixture of protease inhibitors. A large proportion of benzodiazepine binding sites was solubilized in the absence of apparent GABA binding capacity by incubating crude synaptic membrane preparations at 37°C with 0.05% Triton X-100. The characteristics of these two solubilized binding sites resemble those of the membrane-bound binding sites. However, unlike the membrane-bound sites, solubilized GABA and benzodiazepine

recognition sites have lost the ability to cross-react. Hence, solubilized benzodiazepine binding sites are insensitive to GABA stimulation, while solubilized GABA binding sites are no longer protected by the benzodiazepines against heat inactivation. These results indicate that GABA and benzodiazepine recognition sites reside in two different molecules which, when bound to membranes, can interact reciprocally and modulate their binding affinity for specific ligands.

The ligand for the benzodiazepine receptor is probably represented by an endogenous brain peptide (EBP) that we have isolated and partially characterized. The extraction procedure was initiated by homogenizing the rat brain in 20 vol of hot (80°) 1 N acetic acid. After the pH was adjusted to 5, the 48,000 xg supernatant was dialyzed for 24 hrs against 0.1 N acetic acid. The dialyzate was concentrated and applied to a Sephadex G-100 column equilibrated in 0.1 N acetic acid. The fractions were tested for EBP activity. Activity was associated with a protein peak which emerged after ribonuclease A (a marker for 14,000 daltons) and before β-mercaptoethanol (a marker for 78 daltons). An alternative method of extracting the EBP consisted of treating frozen-thawed and well-washed crude synaptic membranes with 0.05% Triton X-100. The suspension was incubated for 30 min at 0°, sonicated for 30 sec and then centrifuged at 100,000 g for 60 min. From this crude extract EBP was isolated by heat treatment at 95°C for 15 min and by chromatographing the subsequent 100,000 g supernatant on a G-100 column. This extraction procedure gives a profile similar to that shown after hot acetic acid extraction. The inhibitory material obtained from G-100 was further purified on a Biogel P-2 column equilibrated in 0.1 N acetic acid. The EBP activity was associated with the protein peak emerging in the void volume and was destroyed by pronase and trypsin but not by phospholipase A_2, C or D. The purified EBP, when subjected to electrophoresis on an SDS-12% acrylamide gel, revealed 2 major bands with molecular weight smaller than 14K. The EBP extract from the BioGel P-2 column was further purified by reverse phase HPLC on a BioRad ODS column with acetonitrile gradient. The protein peak and EBP activity emerged at ~50% acetonitrile. The purified EBP inhibited ^{3}H-diazepam binding competitively. It did not inhibit the binding of ^{3}H-GABA, indicating the EBP can be physically separated from GABA modulin. EBP has no effect on the binding of ^{3}H-cyclohexyladenosine, ^{3}H-QNB, ^{3}H-etorphine or ^{3}H-alprenolol, while it does inhibit effectively ^{3}H-propyl-β-carboline-3-carboxylate binding. EBP displaces ^{3}H-flunitrazepam more effectively from cerebellar membranes than from hippocampal membranes. The amount of EBP that can be extracted from 100 mg of brain is equivalent to 1 pmol of diazepam. With the extraction procedures described above no EBP activity was found in 100 mg of liver and kidney.

In conclusion, our data suggest that BDZ recognition sites are functionally associated with GABA receptors and that an endogenous brain peptide can modulate their function.

The importance of these observations may go beyond the immediate explanation of the mode of action of BDZs and may represent the beginning of a better understanding of the role of GABAergic neurons in anxiety and in other mental disorders. In addition, these observations may help in elucidating details of

the supramolecular nature of postsynaptic GABA and BDZ receptors and also may offer a simple means for studying the nature of endogenous materials involved in anxiety.

Publications:

Massotti, M., and Guidotti, A.: Endogenous regulators of benzodiazepine recognition sites. Life Sci. 27: 847-854, 1980.

Cananzi, A.R., Costa, E., and Guidotti, A.: Potentiation by intraventricular muscimol of the anticonflict effect of benzodiazepines. Brain Res. 196: 447-453, 1980.

Massotti, M., Guidotti, A., and Costa, E.: The regulation of benzodiazepine recognition sites by endogenous modulators. In DiChiara, G., Gessa, G.L., and Costa, E. (Eds): Advances in Biochemical Psychopharmacology, Vol. 26. New York, Raven Press, 1980, pp. 19-26.

Massotti, M., Guidotti, A., and Costa, E.: Purification and characterization of benzodiazepine and gamma aminobutyric acid recognition sites and their endogenous modulators. J. Neurosci. 1: 409-418, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01518-05 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
The study of mammalian GABAergic mechanisms using various methods to measure turnover rates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Khachatrian	Visiting Scientist	SMRP	NIMH
Other:	D. L. Cheney	Chief	SMRP-M	NIMH
	A. V. Revuelta	Visiting Associate	SMRP	NIMH

COOPERATING UNITS (if any)

None

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SECTION
Molecular Pharmacodynamics

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

GABA turnover can be measured by following the conversion rates of ^{13}C glutamate into ^{13}C GABA. This is not possible in cortex, hippocampus, and cerebellum presumably because these contain abundant glutamatergic nerves where ^{13}C glutamate is not converted into GABA. Since it is impossible to correct for this discrepancy, the increment in GABA levels following the intraventricular injection of the GABA transaminase inhibitor, gabaculine and the decrement in GABA levels following subcutaneous injection of the glutamate decarboxylase inhibitor, isoniazid, have been used to estimate GABA turnover rates in various rat brain areas. In those structures where GABA turnover can be measured isotopically, the fractional rate constants measured by perturbing GABA steady state are one-tenth of those measured with the isotopic method. The reasons for the discrepancy between the two methods are being investigated. Meanwhile, the isotopic method and the enzyme inhibitor methods can be used for comparative studies of drug action even though the absolute values obtained are discrepant.

Project Description:

Our objective has been to study the modulation of GABA neurons in various nuclei of rat brain when the trans-synaptic regulation of GABAergic neurons stored in the nucleus is modified.

A recurrent problem in the study of GABA turnover rates is that glutamate functions as an intermediate metabolite in the formation of GABA and as a primary neurotransmitter. Possibly the influence of glutamate as an intermediate metabolite can be minimizing by injecting labeled glucose which is converted more rapidly into glutamate in neurons than in glial cells. It is more difficult to eliminate interferences from glutamate functioning as a transmitter. Only by selecting those tissues where the glutamate that functions as a GABA precursor is rapidly formed from glucose and where its rate of labelling predominates over the other two glutamate pools can the turnover of GABA be adequately estimated. In these tissues we have studied the modulation of GABA neurons by various neurotransmitter systems.

In nucleus accumbens, nucleus caudatus, globus pallidus, substantia nigra, septum and tuberculum olfactorium the rate of ^{13}C-incorporation into glutamate and GABA fulfill the minimal criteria for expression as a precursor-product relationship. In these structures the turnover rate of GABA can be measured by a sensitive gas chromatographic-mass fragmentographic technique involving infusion of ^{13}C-glucose through the tail vein of conscious immobilized animals and measurement of the relative isotopic enrichment attained by the pools of GABA and its immediate precursor, glutamic acid.

However, such a technique cannot be used in deep cerebellar nuclei, cerebellar cortex, colliculus, hippocampus (or hippocampal parts) or in the areas of the cortex (frontal, parietal, occipital or temporal). Thus, in the past we have not been able to use these areas for turnover measurements.

In those areas where the isotopic method does not yield results we have measured turnover using the inhibitors of the enzymes involved in GABA metabolism. Gabaculine, a highly potent irreversible inhibitor of GABA transaminase and isoniazid, a non specific inhibitor of glutamic acid decarboxylase have been used to increase or decrease respectively the GABA levels in various rat brain areas.

The intraventricular injection of gabaculine (5.7 μmol/kg or 15.7 μmol/kg) resulted in a time dependent increase of GABA content in all brain areas studied. This effect was either not evident or minimal during the first 10 min after injection. Following a 10 min delay the GABA content doubled during the next 20 min in cortex and hypothalamus. The GABA content in septum and globus pallidus increased by about 70% and in deep cerebellar nuclei, cerebellar cortex and caudate the GABA content increased by 50-60%. The least pronounced effect of gabaculine was observed in nucleus accumbens (30%). Turnover rates calculated from this method in nmol/mg protein/hr were: hypothalamus, 141; substantia nigra, 84; globus pallidus, 132; septum, 84; deep cerebellar nuclei, 62; cortex, 36; caudate, 30; cerebellar cortex, 22; and accumbens, 14.

Isoniazid (3.3 mmol/kg) injected subcutaneously resulted in a decreased GABA level in substantia nigra, septum, cerebellar cortex and parietal cortex. The reduction was time dependent. The largest effect was observed 40 min after injection at which time convulsions were observed in 60% of the rats. The turnover rates calculated from this method in nmol/mg protein/hr were: substantia nigra, 66; cerebellar cortex, 16; and septum, 49.

The turnover rates measured by gabaculine are one-tenth those measured with the isotope method; those measured with isoniazid are even lower. Perhaps the isotopic method is overestimating the turnover rate of the neuronal pool or perhaps the inhibitors are measuring turnover in a very restricted GABA pool. These data raise several questions: 1) Is transaminase located in glia cells and neurons? 2) Does Gabaculine inhibit GABA transaminase rapidly and irreversibly? 3) Does the accumulation of GABA trigger an inhibitory response in GABAergic cells? 4) Is there a feedback inhibition of GABA synthesis. Regardless of the answers to these questions the isotopic method and the gabaculine/isoniazid methods can be used for comparative studies of drug action even though the absolute values obtained are discrepant. The Gabaculine method can be used to investigate the relationship between neurotransmitters in hippocampus, cortex and cerebellar cortex where GABA turnover rates cannot be calculated using the isotopic method.

The study of the interactions among different neuronal systems in the brain is essential to elucidate the mechanism of action of numerous drugs. Moreover the demonstration of the intricate relationship among the neurotransmitters will contribute to a better understanding of how the diverse systems of the brain response to drug therapy. Such knowledge would provide the background necessary to develop more effective therapeutic approaches.

Publication:

Revuelta, A.V., Cheney, D.L., and Costa, E.: Measurement of γ-aminobutyric acid turnover rates in brain nuclei as an index of interactions between γ-aminobutyric acid and other transmitters. In Di Chiara, G., and Gessa, G.L. (Eds.): Glutamate as a Neurotransmitter. New York, Raven Press, 1981, pp. 169-181.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01520-05 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
The dynamics of catecholamine metabolism in the CNS: Biochemical pharmacology of the spinal cord catecholaminergic neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. Karoum	Staff Fellow	SMRA	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

Adult Psychiatry Branch

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to identify and study the metabolism of catecholamines in spinal cord and to evaluate the factors that influence amine metabolism. Our present interest is to determine the relative rates of dopamine and norepinephrine formation in various regions of the spinal cord.

Project Description:

The catecholamine-containing nerves that innervate the spinal cord originate from specific regions within the brain. Based on the known physiology associated with various regions of the spinal cord and the origin of catecholamine-containing neurons within the brain, it is reasonable to suspect that the rate of catecholamine formation in the cord would vary from region to region. Our objective was to test the hypothesis that catecholamine formation is not uniform in the cord.

Catecholamines and metabolites were analyzed by gas chromatography-mass spectrometry. The rate of catecholamine formation was determined from the rate of their decline after administering alpha-methyl-p-tyrosine methylester to inhibit tyrosine hydroxylase activity. The content of norepineprhine was rather uniform along the cord while dopamine was higher in the rostral portion of the cord than in the caudal portion. For both norepinephrine and dopamine there was a rostrocaudal decrease of their turnover rates along the cord. In the cervical cord, dopamine was formed at a faster rate than norepinephrine. There was no correlation between the content of catecholamine metabolites and amine turnover rates. The non-uniformity of catecholamine turnover in the cord probably arises from the fact that different regions of brain project to different regions of cord, each having a specific physiological function. Furthermore, our study provides added support for the presence of an independent dopamine-containing neuronal system in the spinal cord. Our studies are providing basic information needed to understand spinal cord physiology as well as the pharmacology of drugs that influence catecholamine systems.

Our future studies will be designed to determine whether drugs can modify amine metabolism in some regions of spinal cord and not others. Such studies might provide information about the possible site of action of drugs.

Publication:

Karoum, F., Commissiong, J.W., Neff, N.H., and Wyatt, R.J.: Regional differences in catecholamine formation and metabolism in the rat spinal cord. Brain Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01521-06 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Functional role of substance P in nervous system

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. P. Schwartz	Research Chemist	SMRP	NIMH
Other:	E. Costa	Chief	SMRP	NIMH
	M. Ross	Staff Fellow	SMRP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In studies on the function of substance P and on the effect of drugs on its distribution, the possibility that a pool of substance P precursor exists must be considered. The chick embryo dorsal root ganglion contains a molecular species of high molecular weight immunoreactivity which could function as a precursor in the formation of substance P. The content of this possible precursor is regulated by treatment of ganglia with nerve growth factor. Substance P, and its apparent precursor, have also been found in superior cervical ganglia, probably located in interneurons. In order to carry out pulse chase experiments to demonstrate the nature of the relationship between this possible precursor and substance P, cultures of dissociated neurons from chick embryo dorsal root ganglia have been developed. These cultures are also useful for examining the mechanism of action of drugs such as capsaicin. In parallel similar studies are being carried out in animals treated with capsaicin or exposed to anti-NGF antiserum.

Project Description:

Evidence is accumulating that many peptides, and protein hormones and transmitters are synthesized as inactive precursors, and that the conversion of the precursor to the active component is an important step in the regulation of the modulatory peptide. We have used chick embryo dorsal root ganglia, which contain substance P cell bodies, to search for a precursor to substance P. Incubation of the ganglia in vitro for 20-24 hrs resulted in an increase in the concentration of substance P; this increase was blocked by cycloheximide. In addition, incubation of 9 day chick embryo ganglia with β-nerve growth factor resulted in a further increase in the substance P content of the ganglia, an increase also prevented by cycloheximide. Gel chromatographic analysis of extracts revealed the presence of 2 peaks of substance P-like immunoreactivity (SPLI), one of which eluted at the same position as authentic substance P; the size of the other peak, of a molecular weight larger than substance P, was increased following NGF treatment. We believe this peak may be a precursor to substance P.

In order to study the relationship of the possible precursor to substance P more carefully, by means of pulse labelling and chase experiments, we have been developing a method for culture of dissociated neurons from chick embryo dorsal root ganglia. These neurons can be cultured either with serum or in a chemically defined serum-free medium, in the presence of NGF. The substance P content increases with days in culture. The cultures can be readily treated with drugs and among the studies underway is the effect of capsaicin, a possible neurotoxin which selectively reduces the substance P content. In particular, we will be interested in studying its mechanism of action. Another project will be to determine whether serotonin is present in the ganglia, whether it coexists with substance P in the same neurons, and whether serotonin-depleting drugs will also affect the substance P content.

Another way to study substance P metabolism, as well as development of substance P-containing neurons, is through the use of animals exposed to antiserum against NGF or treated with capsaicin. These two treatments have certain effects in common. In animals exposed in utero or as newborns, there is a loss of substance P-containing cells from sensory ganglia, with a corresponding depletion of substance P in the spinal cord and skin. In adult animals, in contrast, there is a depletion of the substance P content of these tissues with no loss of cell number. The effect of anti-NGF in the adult animals is surprising since sensory ganglia have been thought to lose their NGF responsiveness during embryological development. Studies with the anti-NGF treated animals have shown that substance P-containing neurons in adrenal medulla and ileum are also NGF-responsive, whereas those of the submaxillary gland, the retina, and a variety of brain regions are not.

Publication:

Ross, M., Lofstrandh, S., Gorin, P.D., Johnson, E.M., and Schwartz, J.P.: Use of an experimental autoimmune model to define nerve growth factor dependency of peripheral and central substance P-containing neurons in the rat. Submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01524-06 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Evidence for peripheral dopaminergic neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Z. Lackovic	Visiting Associate	SMRP	NIMH
	M. Relja	Guest Worker	SMRP	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH
	J. Kleinman	Staff Psychiatrist	SMRA	NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine receptors are found throughout the cardiovascular system. Their presence in peripheral structures suggests that peripheral nerves containing dopamine as a transmitter might innervate these receptors. Our objective was to systematically study the catecholamine content of peripheral nerves and organs of animals as well as human tissues taken at autopsy for the presence of dopamine and its metabolites.

Project Description:

There is now evidence for the presence of dopamine receptors throughout the cardiovascular and urogenital systems. These observations are consistent with the notion that there might be a peripheral dopaminergic neuronal system. Our objective was to evaluate whether peripheral nerves contain dopamine and its acidic metabolites in sufficient quantities compared with norepinephrine to suggest that there might be peripheral dopaminergic nerves.

Dopamine and its metabolites and norepinephrine were assayed in human samples obtained at autopsy and animal tissues by gas chromatography mass-spectrometry (GC-MS). GC-MS is the only instrument that will provide high sensitivity together with absolute identification which is essential for this project.

Two observations have been cited in the past as arguments against the possibility that dopamine might be a neurotransmitter in peripheral nerves: first, its low concentration in comparison to norepinephrine; and second, the absence of metabolism in peripheral tissues in vivo. These arguments are not valid for human tissue. We found a rather high dopamine to norepinephrine ratio in most human peripheral nerves and tissue. In genitofemoral and intercostal nerve, dopamine represents more than 40% of the total content of catecholamine. Moreover, some human spinal nerve roots contain more dopamine than norepinephrine. In addition, the concentration of dopamine metabolites in most tissues is higher than in blood and usually higher than the concentration of dopamine. Finally, we have identified a dopamine sensitive adenylate cyclase in the adrenal gland that becomes supersensitive to dopamine after splanchnectomy or treatment with the neurotoxin for catecholamine-containing nerves, 6-hydroxydopamine. Our findings do not elucidate the source and termination of the potential dopamine-containing nerves, however, they suggest an association of peripheral dopamine with the autonomic nervous systems.

Schizophrenia and Parkinsons disease are associated with abnormal metabolism of dopamine. Some of the symptoms of these diseases may be related to a dificiency of peripheral dopaminergic neurons. Moreover, some of the side effects of drugs that act on the central dopaminergic system may be the consequences of actions on peripheral dopaminergic neurons or their receptors. Our studies are an attempt to answer some of these important questions.

Following completion of our present studies we will direct our interest toward establishing the origin and physiological role of the peripheral dopamine.

Publications:

Lackovic, Z., and Neff, N.H.: Evidence for the existence of peripheral dopaminergic neurons. Brain Res. 193: 289-292, 1980.

Lackovic, Z., Relja, M., and Neff, N.H.: Evidence for a peripheral dopaminergic neuronal system. In Usdin, E., Youdim, M.B.H., and Weiner, N. (Eds.): Function

and Regulation of Monoamine Enzymes: Basic and Clinical Aspects. England, MacMillan Press, 1981, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01525-05 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regulation of nerve growth factor content of nervous system-derived tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. P. Schwartz	Research Chemist	SMRP	NIMH
Other:	E. Costa	Chief	SMRP	NIMH
	P. Onali	Visiting Fellow	SMRP	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Nerve growth factor (NGF) is synthesized and secreted by rat C6 glioma, a nervous system-derived cell line. The NGF content of these cells is increased by β-adrenergic agonists, which cause an elevation of cyclic AMP content and the concomitant activation of cAMP-dependent protein kinase. The increase of NGF content does not depend on the increase in protein synthesis which is elicited by isoproterenol, whereas the increase of cyclic nucleotide phosphodiesterase (PDE) does. There are two forms of PDE in the cells; the cyclic AMP-specific form is induced by β-adrenergic agonists, whereas the second form hydrolyzes cAMP or cGMP and is regulated by calcium and calmodulin. The induction of PDE requires translocation of the catalytic subunit of cytosol protein kinase into the nucleus, a process which is inhibited by treatment of the cells with vinblastine or colchicine. Phosphorylation of acidic nuclear proteins is also required for PDE induction; this phosphorylation can be blocked by cordycepin. RNA polymerase II is required for synthesis of PDE mRNA and treatment of the cells with either actinomycin D or α-amanitin inhibits RNA polymerase and blocks PDE induction.

Project Description:

The nervous system-derived cell line C6 glioma synthesizes and secretes NGF. The cells are grown in monolayer culture using standard tissue culture methods. β-NGF has been measured by two different assays: a radioimmunoassay utilizing ^{125}I-NGF and antibody to β-NGF, and a bioassay which measures the outgrowth of neurites of dorsal root ganglia from 9-10 day chick embryos cultivated in presence of various concentrations of β-NGF. The content of β-NGF as well as the amount released in the medium can be increased by treating the cells with agents which increase cyclic AMP content by adenylate cyclase activation or by inhibition of PDE. Incubation of the glioma cells with a β-adrenergic receptor agonist causes an elevation of the cellular cyclic AMP content that is maximal in 15 minutes. Cyclic AMP-dependent protein kinase is maximally activated in 30-60 minutes, and then returns to basal activity by 3 hrs. The glioma cells contain both a cyclic AMP-dependent and -independent protein kinase. The cyclic AMP-dependent form has been characterized as a Type II protein kinase on the basis of its elution pattern from a DEAE-cellulose column using a NaCl gradient.

One consequence of isoproterenol activation of adenylate cyclase in the glioma C6 cells is an induction of cyclic AMP phosphodiesterase (PDE), a process which reaches a peak in 3-4 hrs and requires new protein synthesis. The cell cytoplasm contains 2 forms of PDE, which are separable on a DEAE-Sephacel column. The first form utilizes both cyclic AMP and cyclic GMP as substrates and is activated by calcium and calmodulin. The second from, which acts only on cyclic AMP, is specifically induced by isoproterenol treatment. This form is not affected by either calcium plus calmodulin or cyclic GMP (up to 100 μM). Further purification revealed a single peak of activity with an apparent MW of 54,000, whose specific activity is increased following β-adrenergic stimulation. Kinetic analysis revealed a non-linear Hofstee plot with apparent Km values of 2-5 μM for cyclic AMP.

Characterization of the time course and extent of protein kinase activation is the first step in determining how the cyclic AMP-activated protein kinase is increasing the β-NGF and PDE content of the cells. Following activation of the cyclic AMP-dependent protein kinase, there is a translocation of the catalytic subunit of the kinase from the cytosol to the nucleus. The increased activity can be inhibited by the Type I protein kinase inhibitor specific for cyclic AMP-dependent protein kinase, whereas the protein kinase activity of control nuclei cannot; this supports the concept that it is the catalytic subunit of the cyclic AMP-dependent protein kinase which is translocated into the nucleus following isoproterenol treatment. Pretreatment of glioma cells with vinblastine or colchicine blocks the increase of nuclear protein kinase and the increase of PDE activity elicited by isoproterenol. These results suggest first that the translocation of activated subunits of protein kinase from cytosol to the nucleus is required for the induction of new synthesis of PDE molecules. In addition, since vinblastine and colchicine inhibit microtubule polymerization, the results suggest that microtubules are involved in the translocation process. Regulation of protein phosphorylation depends on activation of protein kinase, location of the activated enzyme, and specific sub-

strates present in the sites where activated enzyme is located. An as yet unidentified nuclear acidic protein(s) represents a substrate for the translocated kinase. At 1-2 hrs following isoproterenol, there is increased phosphorylation of the acidic protein fraction, with no change in the degree of phosphorylation of either histones or the remainder of the nuclear proteins. Both the increased phosphorylation of acidic proteins and the PDE induction can be blocked by cordycepin, suggesting that acidic proteins regulate expression of the gene for PDE. RNA polymerase II is also required for induction of PDE. Its activity in vivo and in vitro as well as the induction of PDE can be blocked by either actinomycin D or α-amanitin.

Since neither cycloheximide nor vinblastine affect the increase in β-NGF content elicited by isoproterenol, it is inferred that at least the early (3 to 6 hr after isoproterenol) increase in NGF is not the result of new protein synthesis. We believe that this increase is the result of the activation of a process whereby pro-β-NGF is converted into NGF. We are currently in the process of isolating pro-β-NGF from mouse submaxillary gland in order to examine more closely the role that the peptidase subunit of 7S NGF plays in the conversion process and the role of the cyclic AMP-dependent protein kinase in activating this process.

Publications:

Schwartz, J.P., and Onali, P.: Regulation of intracellular and nuclear metabolism by stimulation of β-adrenergic receptors on C6 glioma cells. In Usdin, E., Weiner, N. and Youdim, M. (Eds.): Monoamine Enzymes, Second Conference, in press.

Onali, P., Schwartz, J.P., Hanbauer, I., and Costa, E.: Regulation by a β-adrenergic receptor of a Ca^{++}-independent adenosine 3'-5' cyclic monophosphate phosphodiesterase in C6 glioma cells. Biochem. Biophys. Acta, in press.

Schwartz, J.P.: RNA polymerase II in C6 glioma cells: α-Amanitin blockade of cyclic AMP phosphodiesterase induction by β-adrenergic stimulation. Submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01526-05 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Modulation of tyrosine hydroxylase activity in retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Cohen	Staff Fellow	SMRP	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the mechanisms that are responsible for modulating tyrosine hdyroxylase activity in retina. This enzyme is apparently rate limiting for catecholamine biosynthesis. Our present interest is to determine whether tyrosine hydroxylase activity of retina is modified by chronic treatment with neuroleptic drugs.

Project Description:

Dopamine, a putative neurotransmitter in the central nervous system of mammals, is contained in a subpopulation of retinal amacrine neurons. Exposure to light activates retinal tyrosine hydroxylase activity, concomitant with an increase of dopamine synthesis (see Z01 MH 01526-02,03). Activation of the enzyme is characterized by a decrease of the Km for the pteridine cofactor. Neuroleptic drug treatment results in activation of retinal tyrosine hydroxylase activity with a decrease of the Km for the pteridine cofactor also. Our objective was to determine whether chronic treatment with neuroleptic drugs might induce permanent changes of the retinal enzyme.

Animals were placed in a light controlled environment and chronically treated with haloperidol. Enzyme activity was analyzed by standard procedures. Activation of tyrosine hydroxylase failed to occur in animals treated chronically with haloperidol. More than 14 days of treatment were required for the development of tolerance. The tolerance was short lasting. Seventy two hours after the last dose of haloperidol the responsiveness of the enzyme to a single dose of haloperidol returned. Neuroleptic drugs are usually given to humans chronically. Our studies are providing the bases for understanding the toxic and side effects associated with neuroleptic therapy.

Our future efforts will be directed toward studying the effects of neuroleptic drug therapy to pregnant female rats on their offspring. There is now evidence in the literature that the growth and development of brain can be influenced by drugs in utero.

Publication:

Cohen, J., Iuvone, P.M., and Neff, N.H.: Neuroleptic drugs activate tyrosine hydroxylase of retinal amacrine cells. J. Pharmacol. Exp. Ther., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01527-05 SMRP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Studies of endogenous opioids using HPLC

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Meek	Pharmacologist	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Group on High Pressure Liquid Chromatography

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Resolution of neuropeptides by HPLC was studied as a function of mobile phase composition, flow rate, gradient rate, and the size and composition of the peptides themselves. Column efficiency in isocratic separations improved linearly with decreasing flow rate, and decreasing log-molecular weight of peptide. Resolution in gradient separations was primarily a function of gradient rate; flow rate had little effect on resolution, but did affect peak height. The hypothesis that retention of peptides on HPLC could be predicted on the basis of amino acid composition was tested using data form 100 peptides. The high correlation (0.98) indicates that conformation and sequence have minor effects on retention in comparison to the importance of composition.

Project Description:

The separation of peptides by HPLC has become common in this and many other laboratories. Optimum utilization of this separation method requires a knowledge of the factors that affect retention and resolution of peptides. Although it is known that gradient rate and flow rate affect resolution, the exact nature of the relationship has not been clear. One goal of this project was to perform quantitative empirical studies of these variables as well as the influence on resolution of the kind of salts and organic solvents used in the mobile phase. To perform the quantitation, an inexpensive computer based analysis system previously developed in this laboratory was employed (Z01 MH 01527-04 SMRP). The computer allowed precise evaluation of peak shape (variance analysis), peak resolution and timing. The retention of 100 small peptides was measured to determine the extent to which retention depends on amino acid composition.

Main Findings

1) Retention of small peptides (20 residues or less) on reverse phase columns can be predicted by summing the contribution to retention of each amino acid and end group. For 100 peptides, the correlation coefficient (r) was 0.98.
2) Column efficiency in isocratic separations increased linearly with decreasing flow rate and decreasing log-molecular weight of peptide.
3) Resolution in gradient systems improved with decreasing gradient rate, but was only slightly affected by flow rate.
4) Peak height is related to both gradient rate and flow rate.
5) Resolution can be diffusion limiting at low flow rates. Under these conditions, the use of small ($\leq$5u) particles is important with large molecules.

The SIGNIFICANCE of these findings is that they provide basic, systematic data for the experimental variables which must be chosen in the separation of neuropeptides by HPLC. Given the great need for physical separation of these molecules in the study of brain function, these data have filled an acute need in an important area of brain research.

Publications:

Meek, J.L.: Nomogram for adjusting mobile phase composition in reverse phase HPLC. Anal. Chem. 52: 1370-1371, 1980.

Meek, J.L., and Rossetti, Z.L.: Factors affecting retention and resolution of peptides in HPLC. J. Chromatog., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01530-05 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regional distribution and regulation of enkephalin in brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. S. Hong	Staff Fellow	SMRP	NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The hippocampus participates in the regulation of emotions and contains small amounts of met^5-enkephalin but a relatively high amount of receptors. The met-enkephalin content differs in various regions of the hippocampus. The temporal region contains three times more met-enkephalin than the septal region. The CA3 and CA4 regions contain twice the met-enkephalin content of CA1, CA2, and subiculum. The uneven distribution of met-enkephalin within the hippocampus suggests that met-enkephalin neurons may be located in specific sites where they regulate pyramidal cell activity. Studies using both mechanical and chemical lesions suggest that ME containing neurons are short axon interneurons within the hippocampus. The hippocampal ME content increases after the animals exhibit recurrent motor seizures. This result suggests that the met-enkephalin neurons may respond to certain pathological conditions and may be associated with the postictal depression.

Proposed Course:

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01531-04 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Nerve growth factor in human fibroblasts from controls and familial dysautonomia patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. P. Schwartz	Research Chemist	SMRP	NIMH

COOPERATING UNITS (if any)
Department of Human Genetics, Yale University Medical School, New Haven, Connecticut

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Skin fibroblasts from human controls or from patients with familial dysautonomia (FD) have been grown in tissue culture. β-Nerve growth factor (NGF) has been measured in extracts from both type of cells, using a radioimmunoassay and a bioassay. The FD cells have contents of NGF as measured by RIA comparable to the controls. However, the biological activity of the FD samples is about one-tenth that of the controls when expressed as biological units of activity per ng β-NGF as measured in the RIA. Stimulation of β-adrenergic receptors in both cell lines with l-isoproterenol results in a 17-170 fold rise in intracellular cyclic AMP in 10 min. The NGF content of the control fibroblasts increased 50-300% after 3-4 hrs of exposure to isoproterenol whereas there was no change in the NGF content of the FD cells at any time.

Project Description:

Familial dysautonomia (Riley-Day Syndrome, FD) is an autosomal recessive disease, in which patients have impaired autonomic and sensory functions. Symptomatically, these patients resemble animals that have been treated with antiserum to NGF. It has been suggested that there may be an impairment of NGF associated with the disease, and there are reports that FD patients have elevated serum NGF levels.

We have chosen to use skin fibroblasts from FD patients, and fibroblasts from paired controls, to study this problem further. The fibroblasts are grown in monolayer culture, using early passage cells and standard tissue culture procedures. NGF is assayed using a radioimmunoassay which detects β-NGF as well as the β-component of 7S NGF, and also a bioassay in which neurite sprouting from 9-day chick embryo dorsal root ganglia is measured.

NGF remains essentially constant in both control and FD fibroblasts as they go from log to stationary phase growth. Control and FD cells have been found to contain comparable amounts of NGF as measured by radioimmunoassay. However, estimation of the NGF content of the same extracts by a bioassay procedure shows that the extracts from FD cells are much less biologically active than cells from paired controls. When expressed as biological units of NGF per ng β-NGF (as measured in RIA), there is a ten-fold difference in biological activity. In contrast, fibroblasts from patients with dystonia musculorum deformans, another genetically inherited neurological disease, contained levels of biological activity per ng of immunoreactive β-NGF that are similar to those of control extracts. Three dystonia lines, five FD lines and seven control lines have been assayed. Comparable assays of cells from heterozygote parents and their FD children have demonstrated that the parents' cells resemble those of normal controls rather than an intermediate form. Assay of parental cells therefore cannot be used for diagnostic purposes at this point. Because of the increasing use of amniocentesis for prenatal diagnosis, we have been studying the feasibility of growing fibroblasts from aminotic fluid and measuring the NGF content by both radioimmunoassay and bioassay. We have now established a baseline NGF content for normal amniotic fluid cells (~0.2 B.U./ng of β-NGF) and have assayed cells from one pregnant woman at risk; a large number of samples will be required to establish a correlation between the NGF activity of amniotic fluid cells and the presence or absence of familial dysautonomia.

These fibroblasts have a β-adrenergic receptor linked to adenylate cyclase. Stimulation with l-isoproterenol caused a 17- to 170-fold increase of cyclic AMP in all lines tested. In the control lines, NGF content increased 50-300% within 3-4 hrs following isoproterenol. However there was no change in the NGF content of any FD cell line over the course of a 7.5 hr treatment with isoproterenol. These results suggest that there may be a defect in the processing of a pro-β-NGF in familial dysautonomia. The defect could lie in the pro-β-molecule, in the converting peptidase, thought to be the γ-subunit of 7S NGF, or at some other step. Research is now being carried on to characterize the chemical differences between the NGF of control and FD fibroblasts. One

approach has been to develop an isolation procedure for the pro-β-NGF molecule, in order to prepare antibodies against it and thereby develop an assay, as well as to do chemical studies on its structure, for comparison ultimately with the structure of the pro-β-NGF from FD extracts. Submaxillary glands removed from castrated male mice which were treated with testosterone provide a relatively enriched source of the pro-β-NGF. Chromatography of an extract of these glands over a Sephadex G-100 column resulted in fractionation of β-NGF immunoreactivity into two peaks, the first of which is the large MW 7S NGF. Currently we are characterizing the other peak, which has the expected MW for pro-NGF, to determine if incubation with the γ-peptidase can convert it into β-NGF. The alternative approach has been to carry out an immunoprecipitation on the cell extracts with the β-NGF antiserum, separate the immunoprecipitated material on an SDS-polyacrylamide gel, iodinate the NGF band, digest with trypsin and run 2-dimensional peptide maps. The human β-NGF shares 4-5 tryptic peptides (labelled with the 125 iodine) with mouse β-NGF; however, no differences have been detected between the NGF of normal and dysautonomic cells using these particular conditions. These studies are continuing and will be expanded to include pro-β-NGF once the antibody becomes available.

Publication:

Breakefield, X.O., Edelstein, S.B., Grossman, M.H., and Schwartz, J.P.: Variations in MAO and NGF in cultured human skin fibroblasts. In Gershon, G.S., Matthysse, S., Cianarello, R.D. and Breakefield, X.O. (Eds.): Genetic Strategies in Psychobiology and Psychiatry. Calif., Boxwood Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01532-04 SMRP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regulation of catecholamine receptor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. M. Chuang	Chemist	SMRP	NIMH
Other:	L. Farber	Chemist	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

During desensitization of β-adrenergic receptors in frog erythrocytes, the binding sites of this receptor are internalized. Certain classes of gangliosides were found to diminish the internalization of β-receptor recognition sites. These effects can be attributed to the inhibition of the agonist binding to membrane-bound β-receptors, thereby inhibiting the isoproterenol (IP)-sensitive adenylate cyclase. Methylamine and several other inhibitors of transglutaminase prevented the beta-receptor internalization and the loss of membrane-bound beta-receptors elicited by incubation with IP. The potency of these compounds to inhibit the internalization and desensitization of beta-receptors could be correlated with their potency to inhibit the transglutaminase activity in the erythrocyte lysate. Thus, transglutaminase might be essential in triggering the internalization following IP binding to cell surface receptors. Various lysosomal enzymes can be detected in the lysate of frog erythrocytes. Lysosomotropic drugs caused a dose and time dependent decrease in the number of soluble beta-receptors in IP-treated cells. Our results suggest that β-receptor recognition sites may be internalized in coated vesicles and solubilized by limited hydrolysis with lysosomal enzymes.

Project Description

In frog erythrocytes, the desensitization of β-adrenergic receptors is characterized by a decrease in the number of β-adrenergic receptor recognition sites in the plasma membrane and a concomitant increase in the number of these recognition sites in the cytosol. We have documented that this redistribution of the receptor recognition sites reflects the internalization of these sites from the surface into the cell. In order to reach a better understanding of the molecular mechanisms whereby these recognition sites are internalized, we have examined various inhibitors that interfere with this event.

Since gangliosides have been implicated as the receptor for a variety of biological systems, the effect of gangliosides on the internalization of β-receptor recognition sites was examined. Incubation of erythrocytes with gangliosides prior to the addition of isoproterenol was found to inhibit in a dose dependent manner the isoproterenol-elicited receptor internalization. Among the subtypes of gangliosides tested, GT_1 was the most effective. The potency was followed in order by GD_{1b}, GD_{1a}, a ganglioside mixture and GM_1. This potency and rank order can be correlated with those for the inhibition of isoproterenol-sensitive adenylate cyclase. Moreover this cyclase inhibition can be attributed to the interference of the binding of [^{3}H]-hydroxylbenzylisoproterenol, a β-receptor agonist, to β-receptors in isolated erythrocyte membrane. It is of interest that the binding of [^{3}H]-dihydroalprenolol ([^{3}H]-DHA), a β-receptor antagonist, to membrane bound β-adrenergic receptors was much less affected by these gangliosides. Available data are consistent with the view that gangliosides have been incorporated into the plasma membrane in the microenvironment of β-adrenergic receptor; the insertion of this glycolipid in some way interferes with the agonist binding to beta-receptors.

Our previous report has shown that methylamine, an inhibitor of transglutaminase, can prevent the increase of soluble β-receptor binding sites elicited by isoproterenol. Concomitantly the loss of membrane-bound β-adrenergic receptors during desensitization was diminished. We have amplified this investigation by showing that 1) methylamine is equally potent in preventing internalization and desensitization and in inhibiting erythrocyte transglutaminase activity. 2) Among eight inhibitors of transglutaminase examined, the rank order potency for blocking the enzymatic activity is well correlated with that for preventing receptor desensitization and internalization. 3) These drug effects on β-adrenergic receptors are unrelated to the inhibition of isoproterenol-sensitive adenylate cyclase or the binding of [^{3}H]-DHA to β-adrnergic receptors. These results may lend evidence to the view that transglutaminase participates in β-receptor desensitization and internalization. Transglutaminase is proposed to mediate the clustering of ligand-receptor complex in a selective area of plasma membrane termed coated pits. Coated pits eventually invaginate to form intracellular endocytic vesicles which are a vector for the internalized molecules. In light of our results, one may surmise that the internalization of β-receptor recognition sites involves a similar mechanism.

In many systems of protein internalization, the ligands internalized are eventually delivered to lysosomes and degraded there. We have detected the

activity of various lysosomal enzymes in erythrocyte lysate, suggesting the presence of this organelle. We have further examined the effect of various lysosomotropic drugs on the internalization of β-receptor binding sites. Chloroquine, cocaine, tetracaine, tetraethylmethylenediamine and ammonium chloride were found to cause a dose dependent reduction in the number of cytosolic β-receptor binding sites in isoproterenol-treated cells. In contrast, the loss of membrane-bound β-receptor binding sites was not prevented. Moreover the inhibitory effect of lysosomotropic drugs requires the simultaneous presence of isoproterenol and these inhibitors in the incubation medium. Preincubation of cells with these drugs followed by incubation with isoproterenol without the drug did not reduce significantly the number of soluble β-receptor recognition sites. Conversely post-incubation of cells in medium containing these drug did not affect the number the soluble receptor binding sites. These results are compatible with the view that β-receptor recognition sites internalized are delivered to lysosomes and that the recognition sites can be released from some particulate structure, such as receptosomes, by limited hydrolysis with lysosomal enzymes.

Publications:

Chuang, D.M., and Costa, E.: Evidence for internalization of the recognition sites of β-adrenergic receptors during receptor subsensitivity induced by (-)isoproterenol. Proc. Natl. Acad. Sci. USA 76: 3024-3028, 1979.

Chuang, D.M., and Costa, E.: Biochemical sequelae following stimulation with isoproterenol. Neurochem. Res. 4: 777-793, 1979.

Chuang, D.M., Farber, L., Kinnier, W.J., and Costa, E.: Beta-adrenergic receptors from frog erythrocytes: Receptor internalization as a mechanism for receptor desensitization. In Pepeu, G., Kuhar, M.J. and Enna, S.J. (Eds.): Advances in Biochemical Psychopharmacology, Vol. 21. New York, Raven Press, 1980, pp. 143-150.

Chuang, D.M., Kinnier, W.J., Farber, L., and Costa, E.: A biochemical study of receptor internalization during beta-adrenergic receptor desensitization in frog erythrocytes. Mol. Pharmacol. 18: 348-355, 1980.

Chuang, D.M.: Inhibitors of transglutaminase prevent the agonist-mediated internalization and desensitization of β-adrenergic receptors. J. Biol. Chem. (submitted).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01533-04 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Regulation of tissue stores of beta-endorphin-like immunoreactivity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Govoni	Guest Worker	SMRP	NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to understand the involvement of beta-endorphin in the development of obesity, beta-endorphin immunoreactivity was studied in genetically obese male and female mice (C57Bl/SJ ob/ob) and their lean littermate controls (+/?). At three month's of age, the beta-endorphin immunoreactivity in pituitary glands of female obese mice is significantly greater than that of female lean controls. No such difference was observed in male obese and control mice. Since both sexes at three months of age have displayed obvious obesity, the results suggest that increased beta-endorphin may not be directly involved in the development of obesity. The results further suggest that beta-endorphin synthesis in obese mice may be modulated by sex hormones.

Project Description:

Several lines of evidence suggest that beta-endorphin, its precursors, and its metabolites may be involved in the development of obesity. For example, induction of compulsive eating behavior by hypothalamic injection of beta-endorphin, suppression of food intake by naloxone administration, and increased content of pituitary beta-endorphin-like material in genetically obese mice have been observed by various investigators. In order to understand whether the metabolism of beta-endorphin is changed in the pituitary and hypothalamus of obese mice, the hypothalamic and pituitary contents of beta-endorphin and its precursors, proopiocortin and beta-lipotropin were studied in genetically obese male and female mice (C57BL/SJ ob/ob) and their lean litter mates (+/? control). In three month old mice, the total pituitary beta-endorphin immunoreactivity of female obese mice is significantly greater than that of female lean mice; while, in ob/ob and +/? male mice of the same age, no difference was observed. Further analysis of the immunoreactive material revealed that the increased beta-endorphin immunoreactivity is due mainly to an increase in the beta-endorphin content; the beta-lipotropin content appears to be normal. No changes were observed in the beta-endorphin immunoreactivity in hypothalami of ob/ob mice and +/? controls of both sexes. These results clearly suggest that 1) the increase of pituitary beta-endorphin immunoreactivity may not be directly related to obesity, since three month old mice of both sexes have shown obvious obesity; 2) the metabolism of beta-endorphin in obese mice may be modulated by sex hormones, and 3) the obese mice may be an interesting model to study the role of beta-endorphin in peripheral endocrine function.

This project has been terminated.

Publication:

Govoni, S., and Yang, H.-Y.T.: Sex differences in the content of β-endorphin and enkephalin like peptides in the pituitary of obese (ob/ob) mice. J. Neurochem., in press, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01536-03 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Characterization of receptors for putative neurotransmitters

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. C. Olianas	Visiting Associate	SMRP	NIMH
	W. Wojcik	PRAT Fellow	SMRP	NIMH
	P. Potter	Visiting Fellow	SMRP	NIMH
Other:	M. Parenti	Visiting Associate	SMRP	NIMH
	S. Gentleman	Pharmacologist	SMRP	NIMH
	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to identify and study the receptors for putative transmitter substances. Our present objective is to characterize receptor sites in brain using radioligands and to evaluate the factors that modulate neurotransmitter-activated adenylate cyclase activity.

Project Description:

Abnormal receptor function may play a role in the etiology of some forms of mental and neurological diseases. Our objectives are as follows: 1) to study the factors that modify dopamine D-1 and D-2 receptor binding sites; 2) to investigate some of the components involved with adenylate cyclase activation that might be altered when receptor become supersensitive; and 3) to develop a simple reliable assay for cAMP that could be used to determine adenylate cyclase activity.

Dopamine D-1 and D-2 receptor binding sites were studied with radiolabelled dopamine. Dopamine binding sites have been divided into two types on the basis of biochemical and pharmacological evidence. Receptor binding sites linked to adenylate cyclase have been designated as D-1 sites while those not linked to the cyclase have been designated as D-2 sites. We previously reported that ascorbate inhibits the binding of agonist drugs to striatal binding sites (Z01 MH 01536-02 SMRP). We have found that ascorbate inhibits the binding of the natural substrate, dopamine, to both D-1 and D-2 sites of neuronal tissue. These observations have two implications: previous studies of dopamine binding are invalid if ascorbic acid was included in the incubation mixture; and as brain contains one of the highest contents of ascorbic of all tissues it might serve as a modulator of dopamine receptor responsiveness in vivo.

Dopamine receptor responsiveness was evaluated from the activation of adenylate cyclase by dopamine. One of the common features of the neurotransmitter adenylate cyclase receptor systems is the existence of a coupling protein that binds guanine nucleotides. We studied whether the response of striatal adenylate cyclase to guanine nucleotides in the presence of Mn^{++} or Mg^{++} was modified following the development of supersensitivity to dopamine. Supersensitivity was induced by injecting 6-hydroxydopamine (6-OHDA) into the region of the substantia nigra or by making animals morphine dependent.

Both procedures for producing supersensitivity resulted in the same biochemical consequences. Activation of adenylate cyclase was greater in control animals when stimulated with dopamine or guanine nucleotides. Furthermore, Mn^{++} stimulated activity was enhanced in the treated animals as well. Our results support the hypothesis that common biochemical mechanisms are responsible for the dopamine-activated adenylate cyclase supersensitivity induced by denervation with 6-OHDA and morphine dependence. The mechanisms for the development of supersensitivity are unclear at present. It is clear, however, that supersensitivity is the consequence of integrated changes of the receptor complex.

To facilitate our studies of the neurotransmitter adenylate cyclase system we developed a simple rapid fluorometric method for the determination of cAMP. cAMP is converted to a fluorescent derivative, termed etheno-cAMP, by reaction with chloroacetaldehyde for 15 minutes at 100°C. Interfering materials are separated from etheno-cAMP by high pressure liquid chromatography. Our procedure is now routinely used in the laboratory to analyze brain adenylate cyclase activity.

Some forms of mental disease are now thought to be related to abnormal receptor function. In particular, most drugs used to treat schizophrenic interact with dopamine receptors thus implicating dopamine receptor involvement in the disease process. Our studies are providing basic information needed to understand normal physiological mechanisms in addition to the side effect of drugs used to treat these diseases. Future studies will be directed towards understanding the role that adenosine plays in dopamine receptor function as well as the role of neuropeptides in modulating adenylate cyclase responsiveness.

Publications:

Kayaalp, S.O., Rubenstein, J.S., and Neff, N.H.: Inhibition of dopamine D-1 and D-2 binding sites in neuronal tissue by ascorbate. Neuropharmacology 20: 409-410, 1981.

Parenti, M., Gentleman, S., Olianas, M.C., and Neff, N.H.: The dopamine receptor complex: Biochemical consequences of supersensitivity induced by treatment with 6-hydroxydopamine or morphine. In Corsine, G. (Ed.): Clinical Pharmacology of Apomorphine and Other Dopaminomimetics. New York, Raven Press, in press.

Wojcik, W., Olianas, M., Parenti, M., Gentleman, S., and Neff, N.H.: A simple fluorometric method for cAMP: Application to studies of brain adenylate cyclase activity. J. Cyclic Nucleotide Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01537-03 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Biochemical pharmacology of GABA receptor system

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Konkel	Chemist	SMRP	NIMH
Other:	A. Guidotti	Chief	SMRP-N	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Neuroendocrinology

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NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

GABA-modulin, an endogenous membrane protein, which inhibits non competitively ^{3}H-GABA binding to synaptic plasma membranes, has been isolated and purified to homogeneity using acidic extraction followed by Sephadex column purification and HPLC. GABA-modulin is a peptide of 18,000 MW which inhibits the binding of ^{3}H-GABA and the GABA-induced stimulation of ^{3}H-diazepam binding to synaptic membranes. The role of GABA-modulin in the control of GABA receptor system function is presently being investigated.

Project Description:

When GABA receptor sites are occupied by the endogenous agonist the ion channel located in the membrane becomes permeable to Cl^- ion, resulting in a hyperpolarization or depolarization of the receptive neuron depending on the concentration of Cl^- in the surrounding medium. The goal of this project is to provide a better understanding of the function of the GABA receptor complex and in particular the coupling of GABA recognition site with the Cl^- channel as a first step in developing new potent and specific drugs which like benzodiazepines can facilitate the stimulation of GABA receptors.

Our studies indicate that freshly prepared crude synaptic membranes, purified synaptic plasma membranes, or membranes from neuroblastoma clonal cell lines, contain only one population of ^{3}H-GABA recognition sites. Repeated washings of these membranes combined with freezing, thawing and treatment with Triton X-100 unmasks an additional population of GABA recognition sites characterized by high affinity for the agonist. Subjecting the Scatchard plot of these binding studies to the graphic analysis of Rosenthal for one ligand and two types of binding sites, the total density of GABA receptor sites and the relative portion of high (Kd 20-40 nM) and low affinity component (Kd 200-400 nM) of ^{3}H-GABA binding can be estimated.

The increase in number of high affinity binding sites for GABA after Triton X-100 treatment is most likely due to the removal of an endogenous inhibitor protein (GABA-modulin) from the membranes because the increase of ^{3}H-GABA binding is paralleled by a decrease of GABA-modulin content in these membranes. Furthermore the addition of small amounts of purified GABA-modulin to the Triton X-100 treated membranes readily masks the population of receptor with high affinity binding sites.

Using filtration procedures on Biogel P-2 column, Sephadex G-50 and G-100 column, and HPLC, the GABA-modulin has been extracted and purified from synaptic plasma membranes prepared from fresh or microwaved brain. It presents the following characteristics: 1) it is thermolabile; 2) it inhibits high affinity ^{3}H-GABA binding non competitively; 3) it prevents the stimulation of ^{3}H-diazepam binding elicited by GABA; 4) its biological activity is destroyed by trypsin and pronase; and 5) it is not dialyzable.

In agreement with this observation removal of GABA-modulin from synaptic membranes, using Triton X-100, resulted in an increase of membrane phosphorylation. The extent of this increase is proportional to an increment in GABA binding. Both the increase in GABA binding and the increase in protein phosphorylation are nullified by the addition of purified GABA-modulin. These experiments indicate that GABA-modulin may interfere with the function of the GABA receptor unit perhaps by altering the state of membrane phosphorylation.

Evidence has been accumulated to suggest that abnormalities in GABAergic function may be operative in determining the symptoms of Huntington's disease, Parkinson, epilepsy and possibly schizophrenia. Thus the biochemical and

pharmacological characterization of GABA receptors may be a relevant approach to find compounds which are capable of modifying the GABAergic system in man.

We are now developing a simple method to measure GABA-modulin content in different biological samples and to understand whether drugs that interfere with the action of GABA-modulin may be useful in the control of the GABA receptor system in neurological and/or psychiatric disorders.

Publications:

Costa, E., Guidotti, A., Massotti, M., and Mazzari, S.: The regulation of GABA receptors as a possible mechanism for GABA 1,4-benzodiazepine interactions. In Littauer, U.Z., Dudai, Y., Silman, I., Teichberg, V.I., and Vogel, Z. (Eds.): Neurotransmitters and Their Receptors. John Wiley and Sons, Ltd., 1980, pp. 417-427.

Mazzari, S., Leon, A., Massotti, M., Guidotti, A., and Costa, E.: Assaying GABA-modulin activity in brain extracts: Resolution of inherent difficulties. In Yamamura, H., Olsen, L., and Usdin, E. (Eds.): Psychopharmacology and Biochemistry of Neurotransmitter Receptors. Elsevier/North Holland, 1980, pp. 607-615.

Mazzari, S., Massotti, M., Guidotti, A., and Costa, E.: GABA receptors as supramolecular units. In Costa, E., Di.Chiara, G., and Gessa, G.L. (Eds.): Advances in Biochemical Psychopharmacology, Vol. 26. New York, Raven Press, 1980, pp. 1-8.

Massotti, M., Guidotti, A., and Costa, E.: Characterization of benzodiazepine and γ-aminobutyric recognition sites and their endogenous modulators. J. Neurosci. 1: 409-418, 1981.

Massotti, M., Alleva, F.R., Balazs, T., and Guidotti, A.: GABA-benzodiazepine receptors in the offsping of DAMS receiving diazepam: Ontogenetic studies. Neuropharmacology 19: 951-956, 1980.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01538-03 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

The biochemistry of hippocampal synapses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Kinnier	Staff Fellow	SMRP	NIMH
Other:	D. M. Chuang	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
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Dr. D.L. Cheney, Chief of the Section of Molecular Pharmacodynamics, L.P.P.

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The regional distribution of acetylcholine (ACh) and cyclic GMP, ACh turnover rates, ACh fractional rate constant, affinity and number of binding sites for [^{3}H]quinuclidinyl benzylate (QNB) were measured in various regions of the rat hippocampus. In coronal sections of the hippocampus, both ACh and cyclic GMP content were found to be the highest in sections of the amygdaloid end. Within subsectional areas of coronal section of the hippocampus (CA-1, CA-3, and dentate gyrus), the cyclic GMP content, the affinity of [^{3}H]QNB specific binding sites were the highest in the CA-3 and dentate gyrus regions, while the density of [^{3}H]QNB specific binding was the highest in the CA-1 area. In contrast, the fractional rate constant for the efflux of deuretiated ACh was higher in the CA-3 area than in the dentate gyrus. This study demonstrates that an enrichment of ACh content, affinity of [^{3}H]QNB binding sites and cyclic GMP content appears to occur in some specific areas of the hippocampus. These findings suggest that the dissection described in the present experiments defines areas with different degrees of cholinergic innervation. Hence such ACh dissection may be useful to study the cholinergic regulation of the cGMP content of some regions of the hippocampus.

Proposed Course:

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01540-03 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Enkephalin-like peptides of adrenal glands

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Govoni	Guest Worker	SMRP	NIMH
Other:	I. Hanbauer	Pharmacologist		NIH
	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Secretion of enkephalin-like peptides from adrenal glands into circulation was studied in dogs with indwelling cannulae in adrenal vein. The stimulation of the splanchnic nerve induces a voltage dependent increase of met^5-enkephalin immunoreactive peptide (MEIP) in plasma. The MEIP secreted was identified to be met^5-enkephalin using Bio-Gel P-2 column chromatography followed by high pressure liquid chromatography. The effect of splanchnic nerve stimulation was antagonized by hexamethonium suggesting that the release of MEIP was mediated through activation of nicotinic receptor. This suggestion was further supported by the fact that dimethylphenylpiperazinium induced an increase of circulating MEIP. Morphine injection was found to induce a pronounced increase of plasma MEIP in intact dogs but not in splanchnic nerve transected dogs. This effect was blocked by naloxone and also by hexamethonium. The results suggest that the effect of morphine is centrally mediated. This study clearly indicates that enkephalin-like peptides in chromaffin cells of adrenal glands can be secreted into circulation.

Project Description:

The mechanism controlling the in vivo release of met^5-enkephalin immunoreactive peptide (MEIP) was studied. The secretion of MEIP from adrenal glands was studied in the blood withdrawn from the permanently canulated adrenal vein of the dog. In anesthetized dogs, splanchnic nerve stimulation with 20 V, 3 Hz increased MEIP in plasma by 50 to 140% over control value. Through the BioGel P-2 and HPLC, the MEIP secreted was identified to be met^5-enkephalin. The increased secretion of MEIP induced by splanchnic nerve stimulation was markedly blocked by hexamethonium but not totally. In line with this observation, dimethylphenylpiperazinium, a nicotinic agonist, induced both in intact and splanchnic nerve transected dogs an increase of circulating MEIP. Interestingly, hexamethonium and atropine by themselves also reduced the circulating MEIP. The result suggests a possible involvement of chlorinergic system in the physiological release of met^5-enkephalin-like peptide from adrenal glands. Because of the existence of enkephalin-like peptide in the splanchnic nerve, the effect of morphine was tested. Morphine injection induced a very profound increase of plasma level of met^5-enkephalin IP in intact dogs but not in splanchnic nerve transected animals. The effect was blocked by naloxone or hexamethonium. The results suggest that morphine action may involve a centrally mediated reflex eliciting an increase in the release of cholinergic mediation from the splanchnic nerves. The physiological role of enkephalin-like peptide(s) secreted from adrenal glands into the circulation still remain to be explored.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01542-03 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Amino acid neurotransmitters and hippocampal function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Di Lauro	Visiting Associate	SMRP	NIMH
Other:	J. L. Meek	Pharmacologist	SMRP	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Aspartate is an important excitatory transmitter in the brain, as well as a protein component and metabolic intermediate. However, few experimental approaches exist by which the transmitter and metabolic roles can be separated for examination. Using lesion studies, it was shown that aspartate is involved in the perforant pathway to the hippocampus. Since only a few areas of brain have a dense enough innervation by aspartergic neurons for the lesion method to be practical, an aspartate binding method was developed for use in all brain areas. Two binding sites were demonstrated, differing in their sodium dependence, Kd, inhibition by other amino acids, and in their regional distribution. The ability to measure neuronal binding sites (receptors) opens up the possibility of studying interactions with other neurotransmitters, cotransmitters and neuromodulators, as well as the possibility of exploring aspartate pathways in areas of brain where detection of changes in aspartate content would not be possible.

Project Description:

Study of aspartate as a neurotransmitter has been difficult since there are no methods for 1) the histochemical localization of aspartergic neurons, 2) specific measurement of aspartate turnover in the pools that are involved in neurotransmission, and 3) measurement of release without the use of the rather nonspecific radioactive aspartate uptake methods. A few areas of brain (e.g. hippocampus and cochlear nucleus) have sufficiently dense innervation that lesion experiments can demonstrate aspartate loss, and we have succeeded in doing this (Ref. 2). However, binding methods need to be developed that will allow study of all brain areas. We have now suceeded in detecting aspartate receptors on rat brain membranes via measurement of specific high affinity binding of ^{3}H-aspartate.

Major Findings

1) Destruction of the perforant pathway by lesion of the entorhinal cortex causes a temporary decrease in the aspartate content of the rat hippocampus.
2) Two kinds of aspartate receptors can be detected in brain differing in their sodium dependence.
3) The sodium independent form had a low Kd (200 nM), low Bmax (6 pmol/mg) high specificity (not inhibited by D-aspartate or L-glutamate), and decreased in content after destruction of intrinsic neurons with kainic acid. This site is probably a neuronal aspartate receptor.
4) The sodium dependent form had a higher Kd (500 nM) and Bmax (100 pmol/mg), low specificity (inhibited by D-aspartate and L-glutamate) and was unaffected by kainic acid lesions. This binding probably represents an uptake site.
5) The sodium dependent and independent sites differ in their regional distribution; the former is more concentrated in the cerebral cortex, and less in the cerebellum, while the reverse is true for the sodium independent form.
6) Physiological concentrations of calcium and magnesium alter the number of sodium independent binding sites, but do not affect the sodium dependent sites.

Significance

Understanding of brain function requires knowledge of all neurotransmitter systems. Progress has been rapid with the biogenic amines and neuropeptides, where many approaches have been successfully employed for their study. Progress has been much slower with the excitatory amino acids glutamate and aspartate, since techniques available to study them have been severely limited in usefulness. The ability to study aspartate receptors will assist us in learning about the functions of an important component of the nervous system.

Publications:

Schmid, R., Hong, J.S., Meek, J., and Costa, E.: The effect of kainic acid on the hippocampal content of putative transmitter amino acids. Brain Res. 200: 355-362, 1980.

Di Lauro, A., Schmid, R.W., and Meek, J.L.: Is aspartic acid the neurotransmitter of the perforant pathway? Brain Res. 207: 476, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01543-02 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regulation of neurotensin in brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.M. Govoni	Visiting Associate	SMRP	NIMH
Other:	J.S. Hong	Staff Fellow	SMRP	NIMH
	H.-Y.T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Similarities have been shown to exist between Neurotensin (NT) and neuroleptics by other investigators. Intrinsic enkephalinergic neurons in striatum (ST) and in nucleus accumbens (NA) appear to be regulated transynaptically raising the question whether a similar regulation is operative for NT containing neurons which are also present in these areas. Previous results indicated that in rat haloperi-dol injection can increase the of NT selectively in NA and ST. The effect is observed either after acute or repeated treatment. We have now studied the NT content of various brain areas after treatment with different antipsychotic drugs. The results indicate that all the neuroleptics tested (chloropromazine 6.0 mg/kg; Trifluoroperazine, 2.0 mg/kg; Pimozide, 1.5 mg/kg; Haloperidol, 1:mg/kg) increase the content of NT in NA and ST. On the contrary, promazine (10 mg/kg) and Prometazine (25 mg/kg) which are structurally related to phenothiazines but with weak or no neuroleptic activity failed to increase the NT content of NA and ST. This effect is specific for NA and ST and it is not observed in preoptic area hypothalamus, septum, amygdala although these areas are enriched in NT. The Neuroleptics induced increase of NT in NA and ST suggest a modulation of NT metabolism through dopaminergic synapses.

Project Description:

Neurotensin was originally isolated from bovine hypothalamus by Carraway and Leeman (1973). In the periphery neurotensin is found mainly in the gastrointestinal tract; in the brain it displays an uneven distribution with high concentrations in hypothalamic areas and in some nuclei of the limbic system (n. accumbens, amygdala, bed nucleus), lower concentrations of neurotensin are found in cortex, striatum and hippocampus; it is not detectable in the cerebellum. Neurotensin receptors are present in the brain and their distribution parallels that of the peptide itself. These evidences suggest a neurotransmitter or neuromodulator role for neurotensin. Moreover, the intraventricular injection of the peptide produces effects that are some how reminiscent of those induced by neuroleptic injection. In fact, like neuroleptics, neurotensin prolonges the sleeping time after barbiturate administration, impairs the body temperature control, causes muscle relaxation and counteracts, the stereotyped behaviour that follows the intracerebral injection of amphetamine. in a previous study we have found that the acute or repeated adminstration of haloperidol induces an increase of the concentrations of neurotensin in n. accumbens and striatum. The effect is area-specific and it does not occur in any of the other regions tested (septum, hippocampus, hypothalamus, cortex, amygdala). We were interested in determining if such effect was preculiar for haloperidol or it was or if it was a property shared by other neuroleptics. To study this, we analyzed the neurotensin concentrations in various brain areas after repeated treatment (daily injection for two weeks) with chloropramazine (6.0 mg/kg) trifluoroperazine (2.0 mg/kg), pimozide (1.5 mg/kg) and spiroperidol (1.0 mg/kg). All these neuroleptics are able, like haloperidol, to increase the content of neurotensin specifically in n. accumbens and striatum. On the contrary, the concentrations of the peptide were unmodified by the treatment with promazine (10 mg/kg) and prometazine (25 mg/kg), two drugs that are structurally related to phenothiazines, but have little or no neuroleptic activity.

The fact that neurotensin concentrations are affected only in n. accumbens and in striatum, two areas particularly rich in dopaminergic terminals, together with the known ability of neuroleptic to block dopamine receptors might indicate that the metabolism of this peptide is midulated directly or indirectly through dopaminergic synapses.

However the dynamics of the increase of neurotensin content cannot be studied in detail because the levels of the peptide are low and we need first to identify whether there are stores of a neurotensin precursor. The results reported support the hypothesis of a neurotransmitter or neuromodulator role for neurotensin and its involvement in the action of antipsychotic drugs.

The neurotensin over-production triggered by neuroleptics can now be studied with regard to their beneficial actions in the treatement of schizophrenia.

Publications:

Govoni, S., Hong, J.S., Yang, H.-Y.T., and Costa, E.: Increase of neurotensin content elicited by neuroleptics in nucleus accumbens. J. Pharmacol Exp. Ther. 215: 413-417, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01544-02 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Histamine: A possible neuroactive substance in brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Olianas	Visiting Associate	SMRP	NIMH
	A. P. Oliver	Psychologist	SMRA	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

Adult Psychiatry Branch

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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Histamine has been proposed as a neurotransmitter in brain especially in the hippocampus. Our objective is to investigate receptor mechanisms of hippocampus. Histamine-stimulated adenylate cyclase activity of homogenates will be correlated with changes of neuronal firing rates induced by histamine in the isolated hippocampal slice.

Project Description:

There is now substantial evidence that histamine might serve as a neurotransmitter in brain. In peripheral tissues two types of histamine receptors have been identified pharmacologically, H1 and H2 receptors. H2 receptors are coupled to an adenylate cyclase system. Our objective is to correlate, histamine activated adenylate cyclase activity and histamine-induced changes of neuronal firing rates. These studies may reveal a potential role for histamine in brain function.

Histamine activated adenylate cyclase was measured by standard biochemical procedures. Neuronal excitability was measured by recording the frequency of interictal spikes in region CA3 of a hippocampal slice with extracellular electrodes.

Our studies revealed that activation of the cyclase and the increase in firing rate were correlated and that both responses were mediated by an H_2-receptor. These studies provide the first evidence that histamine can modulate neuronal excitability and that modulation is associated with an adenylate cyclase activated event, perhaps phosphorylation of a membrane.

In a separate series of studies we evaluated the consequences of long term treatment of rats with antidepressant drugs on both test parameters. Chronic treatment resulted in about a 100 fold increase in the sensitivity of the slice preparation to respond to histamine. In contrast, the histamine activated adenylate cyclase was unchanged by chronic treatment with the antidepressants. Our studies are providing the first evidence that histamine may play a role in neuronal events and that psychoactive drugs can alter responses to histamine.

Publication:

Olianas, M., Oliver, A.P., and Neff, N.H.: Biochemical and electrophysiological studies on the mechanism of action of typical and atypical antidepressants on the H_2-histamine receptor complex. In Costa, E., and Racagni, G. (Eds.): Advances in Biochemical Psychopharmacology. Raven Press, New York, 1981, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01545-02 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Somatostatin in the rat spinal cord

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Stine	Staff Fellow	SMRP	NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
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Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.95	0.95	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Somatostatin is measured in the rat spinal cord by radioimmunoassay. Levels are higher in more caudal spinal segments, much higher in gray than in white matter, and highest in the dorsal horn. Dorsal root ganglionectomy decreases somatostatin levels in the dorsal cord, supporting the proposed role for this peptide as a primary sensory neurotransmitter or modulator. However, somatostatin levels also decrease both rostral and caudal to spinal transection, indicating the probability of ascending and descending somatostatin pathways within the spinal cord. Met-enkephalin levels were not altered by these surgical procedures and Substance P levels were modified significantly only after dorsal root ganglionectomy.

Project Description:

Somatostatin, the growth hormone release inhibitory factor, has been localized in many areas of the central nervous system both by immunohistochemical methods and by radioimmunoassay. In the spinal cord immunohistochemical distribution of somatostatin reported by other investigators supports a possible role for this peptide as a primary sensory transmitter or neuromodulator: staining of terminals is most dense in the dorsal horn and cell bodies are found in the dorsal root ganglia. However, the quantitative distribution of somatostatin within the cord as well as the effect of spinal transection and dorsal root ganglionectomy on somatostatin levels have not previously been reported.

We are interested in the function of somatostatin in the rat spinal cord. Initially we studied rostrocaudal and dorsoventral somatostatin distribution quantitatively by radioimmunoassay. Somatostatin levels are slightly higher in more caudal segments of the spinal cord. Somatostatin levels are highest in the dorsal horn and lowest in the ventral white matter. This is consistent with previous immunohistochemistry work and also with a possible role for somatostatin is a primary sensory transmitter.

To further test this hypothesis, dorsal root ganglia of rats were removed unilaterally from spinal segments caudal to L-4. After 10 days somatostatin levels decreased in the dorsal cord on the operated side but not in other regions. This also supports a role for somatostatin as a primary sensory transmitter.

However, after spinal transection at the T-4 level in the rat, somatostatin levels also decreased both rostral and caudal to the transection. There was no change in brain somatostatin levels. This indicates the likely presence of ascending and descending somatostatin pathways within the rat spinal cord in addition to the proposed primary sensory pathway.

To compare these results with two other peptides similarly distributed within the cord, met-enkephalin and substance P, and also to control for possible non specific effects of the lesions, these peptides were also measured by radioimmunoassay in both ganglionectomy and spinal transection experiments. The levels of met enkephalin, a peptide considered to be localized in interneurons, did not change due to these experimental manipulations. Substance P, which is also proposed as a primary sensory transmitter was found to decrease in the ipsilateral dorsal cord after ganglionectomy. Relative to somatostatin, substance P showed a larger percent decrease after ganglionectomy. However, after spinal transection, this peptide showed only a tendency to decrease which approached statistical significance in areas caudal to the transection.

As a next step in elucidating the function of somatostatin in the spinal cord as well as its interaction with other putative spinal cord transmitters and neuromodulators, we are currently beginning to measure release of the abovementioned peptides from rat spinal cord slices.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01546-02 SMRP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Stimulation and homologous desensitization of cGMP synthesis by opioid, H1 histaminergic and muscarinic cholinergic agonists in N4TG1 cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Gwynn	Guest Worker	SMRP	NIMH
Other:	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

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SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The neuroblastoma clone N4TG1 possesses specific recognition sites for the putative neuromodulators histamine, acetylcholine and enkephalin. These sites are positively coupled with guanylate cyclase: agonist stimulation in the presence of a phosphodiesterase inhibitor causes a dose-related increase in cellular cyclic GMP (cGMP) content. A marked refractoriness or desensitization of this response develops after short-term agonist exposure. In each case this desensitization is specific or homologous and is not mediated by a decrease in either the binding affinity or number of recognition sites. Agonist-induced desensitization of cGMP formation does not, furthermore, appear to be mediated by alterations in the specific activity of either phosphodiesterase or guanylate cyclase. Rather, the marked increase in the activation constant for cGMP formation in desensitized cells indicates that a change in the coupling function(s) between recognition site occupancy and enzyme stimulation has occurred. We are currently examining the roles of membrane calcium channels and phospholipid turnover in desensitization of cGMP synthesis in N4TG1 cells.

Project Description:

Acute Effects

Opiates. ^{3}H-Etorphine binds to intact N4TG1 cells incubatedf at 37 C for 25 minutes with a biphasic kinetic profile: Kd = 1.4 and 7.6 nM; Bmax = 86 and 150 fmol/mg protein. The EC_{50} of etorphine for the stimulation of cGMP formation is 9 nM. The rank order of potency for binding and cyclase stimulation of 7 opiate receptor agonists is identical and consistent with the view that the opiate receptor located on the membrane of N4TG1 is a delta receptor.

Histamine. The high affinity binding of histamine receptor agonists and antagonists is consistent with the conclusion that the histamine receptor is H_1. ^{3}H-Pyrilamine binds to intact N4TG1 cells with a Kd of 3.7 nM and a Bmax of 290 fmoles/mg prot. The EC_{50} for cyclase activation with histamine is $6.4x10^{-6}$M. This effect is antagonized by pyrilamine but not cimetidine.

Acetylcholine. ^{3}H-QNB binds to intact cells with a Kd of 45 and 777 pM for high and low affinity binding sites, respectively. The Bmax of the two receptor populations is 24 and 46 fmol/mg protein. The EC_{50} of carbachol for activation of guanylate cyclase is $6.2x10^{-5}$M. This effect is antagonized by atropine but not by the nicotinic antagonist d-tubocurarine.

Desensitization Studies

Desensitization is described as an agonist-mediated process that significantly increases the EC_{50} and decreases the maximal efficacy of agonists for guanylate cyclase stimulation, while leaving the binding properties of agonists unchanged. Opioid desensitization is stereoselective, inhibited by naloxone, homologous and affects the responsiveness of the amplifying system (guanylate cyclase). Our studies show that changes in the specific activity of guanylate cyclase or phosphodiesterase do not underlie this desensitization.

The observed increase (4-12 fold) in the activation constant (Ka) for stimulation of cGMP formation in desensitized cells indicates that a change in the coupling function between binding site occupancy and enzyme stimulation has occurred. We are now investigating the roles of membrane calcium channels and phospholipid turnover in opioid-induced desensitization of cGMP formation in N4TG1 cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01547-02 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Fate of enkephalin-like peptide released from adrenal gland

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Harsing	Visiting Associate	SMRP	NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several lines of evidence indicate that enkephalin-like peptides in adrenal gland can be released into circulation. In order to explore the role of these released peptides, the stability of the enkephalin-like peptides extracted from adrenal glands in plasma was studied. The high molecular weight enkephalin-like peptides were found to be very stable, while, the low molecular weight enkephalin-like peptides were rapidly degraded in plasma. Our preliminary result also suggests that there may be an enkephalin binding peptide or protein in plasma. Hence it is possible that enkephalin may be released into circulation in some kind of protected form such as bound to a large peptide or protein. In fact presence of enkephalin in blood has been documented in spite of its very short half-life. We are planning to explore the possible target tissues for both high and low molecular weight forms of enkephalin-like peptides in periphery.

Proposed Course:

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01548-02 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Afferent control of serotonergic pathways

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. M. Forchetti	Guest Worker	SMRP	NIMH
Other:	J. L. Meek	Pharmacologist	SMRP	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The afferents to serotonin (5HT) containing cells of the median raphe nucleus are known to include GABA, substance P and met enkephalin. The effect of injecting these substances into the median raphe nucleus was studied on serotonin turnover. GABA had a tonic inhibitory effect on 5HT activity; substance P and met enkephalin both had an excitatory effect on 5HT activity. Since GABA interneurons might be involved in control of serotonergic activity, we also examined the effect of substance P on the turnover of GABA in the median raphe nucleus. As an estimation of GABA turnover, we measured the accumulation of GABA after inhibition of its catabolism with gabaculine. Stereotaxic injections of this drug in the median raphe caused an increase in GABA that was linear with time for up to 90 min, and rapid in onset. Substance P injection into the median raphe increased the rate of accumulation of GABA by 30%. The data suggest that the predominant effect of substance P is directly on 5HT cell bodies, and does not act via a GABA interneuron.

Project Description:

We have previously found that GABA has a tonic inhibitory effect on the median raphe nucleus (Ref. 1). We wished to extend our studies to two other transmitters known to occur in the raphe (substance P and met enkephalin), and also to examine whether effects on serotonin turnover might be mediated by GABA interneurons. GABA is an important inhibitory transmitter whose turnover has been measured by stable isotope incorporation methods. However, this approach is impossible in some brain areas due to difficulties in measuring the specific activity of the actual precursor pool. Approaches involving peripheral injection of drugs to block GABA synthesis or breakdown have also had limited success since the drugs may cause side effects like convulsion or sedation, may block both synthesis and breakdown of GABA, and may have slow onsets of action. We therefore studied 5HT and GABA turnover in the raphe after local injection of substance P and a metabolically stable met enkephalin analog. To estimate GABA turnover, we measured the change in GABA content after injection of isoniazid or gabaculine into the median raphe to block synthesis or degradation, respectively.

Major findings

1) Turnover of 5HT is under tonic inhibitory GABA-ergic control. GABA agonists increase 5HT turnover, GABA antagonists decrease 5HT turnover.
2) 5HT cells are activated by local injection of substance P.
3) 5HT cells are also activated by D-Ala-methionine enkephalin amide.
4) Injection of an irreversible GABA transaminase inhibitor (gabaculine) to block GABA catabolism causes an increase in GABA content that is rapid in onset and linear for several hours after injection.
5) Injection of substance P into the median raphe causes a significant increase in the gabaculine induced GABA accumulation.
6) Injection of a GABA synthesis blocker (isoniazid) caused a rapid decrease in GABA content, but the decrease was not exponential, and was brief in duration.
7) Lesion of the striato-nigral pathway caused a 80% decrease in GABA content of the substantia nigra, and an 80% decrease in the gabaculine induced GABA accumulation.
8) The GABA which accumulates after gabaculine is probably not physiologically active since it does not mimic the effect on serotonergic activity of injection of the GABA agonist muscimol.
9) The approach of local injection of gabaculine to irreversibly inhibit GABA breakdown has the advantage over the intraperitoneal or intraventricular route of less possibility of interference due to effects on other pathways, lower toxicity, more rapid onset of action, linear initial rates of accumulation, and greater possibilities of control of concentration in discrete areas.

Significance

New approaches to study GABA should aid us in our understanding of this important inhibitory transmitter. While it remains to be seen whether the

estimate of GABA turnover obtained with the use of gabaculine is quantitatively correct, the technique should prove very useful in study of the interaction of GABA with other transmitters. Therapeutically important drugs such as the benzodiazepines (valium) work via GABAergic mechanisms, so studies of how these drugs affect GABA turnover in discrete nuclei may help us to understand some of the details of how these drugs affect brain function.

Publications:

Forchetti, C.M., and Meek, J.L.: Evidence for a tonic GABAergic control of serotonin neurons in the median raphe nucleus. Brain Res. 206: 208-212, 1981.

Forchetti, C.M., and Meek, J.L.: Neurotransmitter interactions in the median raphe nucleus. In Usdin, E., Youdim, M.B.H. and Weiner, N. (Eds.): Structure and Function of Monoamine Enzymes, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01549-02 SMRP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Regulation of imipramine binding sites in rat brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Brunello	Visiting Associate	SMRP	NIMH
Other:	W. J. Kinnier	Staff Fellow	SMRP	NIMH
	D. M. Chuang	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A high affinity, saturable and stereospecific [^{3}H-] imipramine binding to crude synaptic membranes preparations from hippocampus is sodium dependent and inactivated rapidly at 37 °C by a Ca^{2+}-dependent protease. This recognition site is sensitive to phospholipase A_2 and pronase and can be solubilized by Triton X-100. These binding sites are also present in neuroblastoma cell cultures. Many other typical antidepressants but not the atypical antidepressants can displace ^{3}H-imipramine bound to synaptic membranes. Mianserin, an atypical antidepressant, also binds to specific synaptic recognition sites. Destruction of 5HT neurons by intracerebral injection of 5,7 dihydroxytryptamine reduced the number of imipramine binding sites in several brain areas, whereas the number of mianserin binding was increased. Depletion of 5HT by p-chlorophenylalanine increased the mianserin binding sites without modifying the characteristics of imipramine binding. Thus some imipramine binding sites are located on serotonergic terminals, while some mianserin binding sites could be associated with post-synaptic serotonergic receptors. Available evidence also suggests that the binding sites of imipramine and those of mianserin are not exclusively associated with only one biochemical type of synapse.

Project Description:

Brain membranes possess sites which bind typical tricyclic antidepressants with high affinity (Nature 281:148, 1979); though these binding sites can not be identified as recognition sites for known neurotransmitters they may be directly related to the site of action of endogenous modulators of synaptic activity, such as cotransmitters. We have previously shown that in rats receiving repeated injections of imipramine for at least 14 days, there is a reduction in the number of the high affinity binding sites for [^{3}H]-imipramine. To reach a better understanding of the molecular mechanisms involved in the therapeutic action of imipramine in relieving depression and in the hope to elucidate valid biochemical markers of effective disorders, we are characterizing the imipramine binding sites in crude synaptic membranes prepared from rat hippocampus and other rat brain structures. The binding of ^{3}H-imipramine to hippocampal membranes has a Kd of 8.3±1.5 nM, Bmax of 800±60 fmol/mg protein and a Hill coefficient of 1.0. The number of specific binding sites for ^{3}H-imipramine was sodium dependent and was rapidly and irreversibly reduced when the incubation temperature was raised from 0°C to 37°C. Experiments using leupeptin and EGTA suggest that this temperature-dependent inactivation is due to a destruction of the binding sites by Ca^{2+}-dependent proteases released upon disruption of the cells. The binding of ^{3}H-imipramine to hippocampal membrane is also sensitive to phospholipase A_2 as well as pronase, indicating that the regulation of this recognition sites is complex. The ^{3}H-imipramine binding sites can be solubilized from membranes by treatment with Triton X-100. However, when the binding sites are solubilized they display a high level of nonspecific binding. Studies of tissue distribution and subcellular localization of the imipramine binding sites suggest that these sites are located in neurons. This possibility is strengthened by the finding that the high affinity imipramine binding sites are also detected in the membranes of neuroblastoma N4TG1 cells.

Recently a high affinity binding of ^{3}H-mianserin, an atypical antidepressant with serotonin receptor blocking activity, has been described in membranes from brain cortex. We have investigated the characteristics of mianserin and imipramine recognition sites and the possible involvement of the serotonergic system in their regulation. Imipramine binding was down-regulated by chronic treatment with desmethylimipramine (DMI) (10 mg/kg i.p. for 20 days), in several brain areas but it was unaffected by a similar treatment with mianserin. On the other hand mianserin binding was not down-regulated by a chronic treatment with either mianserin or DMI. Destruction of 5HT neurons by intracerebrally injected 5,7 dihydroxytryptamine led to a reduction (by about 60%) in the number of imipramine binding sites without changes in their affinity, whereas the number of mianserin binding sites was doubled. Depletion of 5HT stores following 15 days treatment with p-chlorophenylalanine caused an increase in the number of mianserin binding sites but failed to change the characteristics of imipramine binding. From these data we infer that the recognition sites for the two drugs are different and suggest that some of the imipramine binding sites may be located on serotonergic axon terminals, while the mianserin sites are associated with postsynaptic serotonergic receptors. Moreover, the evidence obtained suggests that neither of these binding sites is

related to a biochemically monospecific synaptic system. The information we obtained has gained some insight of the molecular mechanism involved in the therapeutic actions of these typical and atypical antidepressant drugs.

Publications:

Kinnier, W.J., Chuang, D.M., and Costa, E.: Down regulation of dihydroalprenolol and imipramine binding sites in brain of rats repeated treated with imipramine. Eur. J. Pharmacol. 67: 289-294, 1980.

Kinnier, W.J., Chuang, D.M., Gwynn, G., and Costa, E.: Characterization and regulation of high affinity [^{3}H]-imipramine binding to rat hippocampal membranes. Neuropharmacology, in press, 1981.

Brunello, N., Chuang, D.M., and Costa, E.: Difference in the synaptic location of the high affinity sites that bind imipramine and mianserin. Submitted to Science.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01550-01 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Biochemical mechanisms regulated by dopamine D-2 receptors in anterior pituitary

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Onali	Visiting Associate	SMRP	NIMH
Other:	J. P. Schwartz	Research Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The vasoactive intestinal peptide (VIP) stimulates prolactin release and adenylate cyclase (a.c.) activity in anterior pituitary. Since dopamine (DA) inhibits prolactin secretion by acting on D_2 receptors, we investigated whether it changed the VIP activation of pituitary a.c. DA failed to affect basal a.c. activity at a concentration as high as 10^{-4} M, but inhibited the a.c. activation elicited by VIP. The inhibition was dose-dependent and reached a maximum (50-60% reduction of the cAMP formation stimulated by 10^{-7} M VIP) at a concentration of 10^{-5} M. d-Butaclamol (1 μM) but not l-butaclamol, completely blocked the dopamine inhibition while neither drug affected the a.c. activation by VIP. Apomorphine, a dopaminergic agonist, mimicked the effect of DA, whereas l-phenylephrine and l-isoproterenol were ineffective at concentrations up to 10^{-5} M. The results suggest that activation of D_2 receptors of anterior pituitary can curtail the a.c. activation elicited by VIP. The pharmacological specificity of this D_2 response is currently under investigation.

Project Description:

Biochemical and pharmacological data have demonstrated the existence of multiple receptors for dopamine (DA). A current classification distinguishes two classes of DA receptors: the D-1 receptors which are coupled to adenylate cyclase and the D-2 receptors whose stimulation does not increase cAMP formation in tissues. The D-2 receptors were also identified on the basis of their high sensitivity to DA and DA agonists like ergot alkaloids and their susceptibility to specific antagonists such as sulpiride and metoclopramide. The DA receptors in anterior pituitary are considered a prototype of D-2 receptors. Their stimulation causes a marked inhibition of prolactin release both in vivo and in vitro. Both the biological events involved in this inhibitory effect of DA as well as the biochemical mechanisms regulated by the D-2 receptors are still poorly understood. If one accepts the theory that cyclic AMP mediates the release of pituitary hormones, then the inhibitory effect of DA could be mediated by a decrease in cyclic AMP formation in the mammatrophs. However, there are conflicting reports in the literature regarding the ability of DA to lower cAMP levels in the pituitary gland. Furthermore, investigators have found stimulatory, inhibitory and no effect of DA on the adenylate cyclase activity of pituitary gland. This discrepancy may be partially due to different experimental conditions and to different source of the tissue, since human pituitary adenomas, whole rat pituitary gland and rat anterior pituitary have been used. The pituitary contains a heterogeneous cell population and, therefore, an effect on the adenylate cyclase activity of mammotrophs could be easily masked by the high background due to the activity of the enzyme present in other cell types.

Recently it has been shown that the vasoactive intestinal peptide (VIP) is a potent activator of adenylate cyclase in anterior pituitary. In vitro, this peptide stimulates selectively the release of prolactin. Since biological effects of VIP are limited to the mammotrophs, it is likely that the VIP-stimulated adenylate cyclase activity is also an expression of the enzyme activity present in this cell type. Therefore the VIP-stimulated adenylate cyclase activity could represent a model for studying the coupling of dopamine receptors of mammotrophs with this enzyme system. As an initial approach, we investigated the effect of dopamine on VIP-stimulated adenylate cyclase activity in homogenates of rat anterior pituitary gland. We found that DA failed to affect basal enzyme activity, but inhibited the stimulation of adenyl cyclase elicited by VIP. This inhibition was dose-dependent with an IC_{50} value of approx. 4×10^{-7} M. The maximal inhibition was obtained with 10^{-5} M DA and was equal to a 50-60% reduction of the VIP stimulation. DA agonists like apomorphine, A, 6-7,DTN and bromocryptine mimicked the effect of DA. The inhibitory effect of DA was reversed by dopamine antagonists according to their stereospecificity for the DA receptor. (-)Sulpiride, a specific D-2 blocker, was a potent antagonist of the DA effect. DA had no effect on the adenylate cyclase activity stimulated by prostaglandin E_1, a hormone which does not release prolactin. The results indicate that D-2 receptors in anterior pituitary are coupled to adenylate cyclase in an inhibitory manner. By this mechanism dopamine may modulate the sensitivity of the cAMP generating system to secretagogues like VIP. Further studies using primary culture of anterior pituitary

cells are in progress in order to correlate these biochemical changes with the pattern of prolactin release. Moreover, we are interested in investigating the biochemical events responsible for the inhibitory coupling of D-2 receptors with adenylate cyclase. The opposite nature of the coupling of D-1 and D-2 receptors to adenylate cyclase may mediate different biological responses.

Publication:

Onali, P., Schwartz, J.P., and Costa, E.: Stimulation of dopamine receptors inhibits vasoactive intestinal peptide stimulation of adenylate cyclase in rat anterior pituitary. Proc. Natl. Acad. Sci. USA, submitted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01551-01 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Is insulin a neuromodulator in the central nervous system?

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. L. Barbaccia	Visiting Fellow	SMRP	NIMH
Other:	D. M. Chuang	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Rat brain contains insulin-immunoreactive material and insulin binding sites. The insulin and binding site content shows regional distribution; it is high in the olfactory bulb. Using slices of the olfactory bulb we have found that insulin like dopamine (DA) can stimulate an accumulation of cAMP. Although DA and insulin enhance the cAMP content when given separately, they can no longer act when given together. Haloperidol, which can partially block the DA effect, is ineffective on insulin-dependent cAMP accumulation. Sulpiride, which fails to change the basal cAMP levels, curtails the cAMP accumulation elicited by insulin. Since internalization of insulin may be a mechanism that explain interactions between DA and insulin in olfactory bulb, we have studied insulin internalization using frog erythrocytes as a model system. Our results show that after 30 minutes of incubation at 30°C about 80% of insulin is tenaciously bound to the cells and cannot be removed by procedures which detach the hormone bound to the cell surface, suggesting that insulin internalization has occurred. This tenacious linkage is temperature dependent and requires metabolic energy. Studies are in progress to elucidate the functional significance of this insulin internalization.

Project Description:

The content of insulin-like material and the density of insulin binding sites are unevenly distributed in the central nervous system (CNS). Insulin content seems to be unrelated with the presence of blood, as Howrankova and coworkers reported (PNAS 75:5737, 1978).

Although the molecular mechanisms whereby insulin acts have been extensively studied, little is known about the function of insulin in CNS. Our working hypothesis was that insulin in brain may modulate synaptic function. Since olfactory bulb is one of the areas richest in insulin and contains high density of binding sites, we have used this brain region as a model system to study whether insulin modifies central synaptic mechanisms.

Since immunohistochemistry has shown that olfactory bulb contains dopaminergic cell bodies located among the periglomerular and tufted cells, we have examined whether olfactory bulb contains dopamine (DA)-dependent adenylate cyclase. Using 300 μ slices of olfactory bulb which were preincubated for 30 minutes was studied whether DA can increase the cAMP content in 10 minutes. DA in concentration $3x10^{-5}$M or greater increased the cAMP content of olfactory bulb slices. Monolateral bulbectomy increased the responsiveness of adenylate cyclase in the contralateral olfactory bulb to DA stimulation. The cAMP accumulation elicited by DA was partially inhibited by haloperidol ($5x10^{-6}$M), while was facilitated by sulpiride ($5x10^{-6}$M) and IBMX ($5x10^{-6}$M). Insulin, which by itself did not affect the cAMP content when added to slices of frontal cortex and striatum, was also able to increase in a concentration depending manner (from $3x10^{-7}$M to $1.8x10^{-6}$M) the cAMP content of olfactory bulb slices. This insulin action was unaffected by haloperidol ($5x10^{-6}$M), but was facilitated by sulpiride ($5x10^{-6}$M) and IBMX ($5x10^{-6}$M). Monolateral bulbectomy facilitated the responsiveness in contralateral bulb to insulin in terms of cAMP accumulation. Moreover, when the maximal effective doses of DA (10^{-4}M) and insulin (10^{-6}M) were applied together in the same olfactory bulb slices preparation we would no longer detect the increase of cAMP content elicited by these two hormones as when given separately. Thus our results suggest that insulin and DA are interacting in the olfactory bulb of rat. The neuronal location of insulin in olfactory bulb and the molecular mechanisms whereby dopaminergic receptors and insulin receptors interreact are currently under our investigation.

It has been shown in different systems that insulin can be internalized following its binding to cell surface receptors. It has also been speculated that this insulin internalization may be essential for some long term action of the hormone such as enhanced gene expression. Also in brain the action of insulin may require internalization. We used the frog erythrocytes as a model system to characterize these "post-receptor" events. We found that the frog erythrocytes can bind insulin in a temperature-dependent manner; the binding is biphasic at 30°C and monophasic at 4°C. At 30°C the two binding sites have a Kd of 0.6 and 18 nM, respectively; at 4°C the Kd is 22 nM. Incubation of erythrocytes for 1 h at 30°C with different, concentration of insulin (33, 330 nM) caused a decrease in Bmax by about 30% with no change in Kd. The insulin

specifically bound to frog erythrocytes at 4°C could be removed by treatment with 0.2N acetic acid/0.5M NaCl. In contrast after 30 minutes of incubation at 30°C the insulin was incorporated into the erythrocytes by a different mechanism and could not be released anymore by the above mentioned procedure. Since this mild acid treatment has been shown to remove selectively the ligand bound to surface receptors, the tenacious binding may therefore reflect the internalization of insulin molecules. The insulin internalization occurring at 30°C could be inhibited by dinitrophenol in a dose dependent manner, suggesting the involvement of energy-requiring steps. Both the detector populations found at 30°C seem to be involved in the internalization of insulin. It is now important to characterize the insulin internalization. We plan to pursue this goal isotologically and biochemically. Biochemically we have used Percoll continuous gradient and gel filtration. The results suggest that the insulin internalized is partially associated with lysosomes and it might be degraded by lysosomal enzymes. Now we are studying whether degradation products of internalized insulin have a physiological role. There is evidence suggesting that in hepatocytes a small molecule, probably deriving from proteolytic degradation of internalized insulin, is effective in enhancing mRNA synthesis in isolated nuclei; however intact insulin per se was ineffective in causing this event.

In summary the aim of this project is to study the role of insulin as a brain cotransmitter modulating the synaptic transmission of other known messengers.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01552-01 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Agonist and antagonist of benzodiazepine receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. G. Corda	Guest Worker	SMRP	NIMH
Other:	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Drugs binding to a recognition sites for endogenous ligands can act as agonists and antagonists. An imidazobenzodiazepinone derivative, Ro 151788 is the first representative of specific benzodiazepine antagonists. However since anxiolytic benzodiazepines probably act as antagonists the RO 151788 may be acting as an agonist, that is it mimics the endogenous agonist. Studies with isoniazid show that benzodiazepines antagonize the convulsant action of isoniazid doses that deplete brain GABA by about 50%, in contrast RO 151788 facilitate these convulsions.

Project Description:

This series of investigation are directed to evaluate the pharmacological profile of an imidazobenzodiazepinone (RO 151788) and to predict possible clinical applications of the compound. It includes essentially three approaches: pharmacological, psychopharmacological and biochemical.

A) Pharmacological. Doses of 400 mg/kg i.p. of isoniazid cause a recurrent Jaksomian like to tonic-clonic convulsion episodes after a latency time of 30 to 50 minutes. When RO 151788 is injected (5 to 10 mg/kg) at 25 minuts after the injection the number, duration and severity of convulsant episoides increases dramatically. In contrast when RO 151788 is omitted and it is substituted with one of diazepam (1 to 3 mg/kg i.p.) the severity, intensity and number of convulsions is dramatically decreased. This data is consistent with the view that binding of diazepam to the benzodiazepine receptors maximizes the action of GABA at specific brain receptors whereas the binding of the benzodiazepine antagonist RO 151788 minimizes GABA action on receptors. When the isoniazid convulsiveness explodes at 30 minutes post injection, there is a depletion of GABA content in substantia nigra, cerebellum and hippocampus of about 50%.

B) Psychopharmacological. Bilateral ablation of olfactory bulbs cause the appearance of a number of behavioral changes. Among others the rats made thirsty by withdrawing water from about 70 hrs, persist in drinking though they are repeatedly shocked on approaching the water. This syndrome resembles very much benzodiazepine action, in fact diazepam treated rats obstinately drink even though they are repeatedly shocked. It will be tested whether RO 151788 antagonizes the obstinate drinking of water by thirsty rats caused by bilateral bulbectomy.

C) Biochemical. It is known that in cerebellum slices the addition of benzodiazepine or GABA mimetics cause a decrease of cGMP content. The possibility that RO 151788 enhances cGMP content and particularly facilitates the decrease in cerebellar cGMP elicited by glutamate. It will be studied whether this action of RO 151788 is antagonized by GABA receptor partial agonists or by GABA receptor antagonist.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01553-01 SMRP

PERIOD COVERED
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)
Biosynthesis of met^5- and leu^5-enkephalins in bovine adrenal medulla

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. Lindberg	Guest Worker	SMRP	NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.95	0.95	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study, the formation of met^5- and leu^5-enkephalins from high molecular weight enkephalin-like peptides was investigated. A soluble protein fraction prepared from bovine adrenal chromaffin granules was found to produce low molecular weight enkephalin-like peptides (LMW enk-like) when incubated at 37°C. The LMW enk-like peptides include met^5- and leu^5-enkephalins and other immunoreactive peptides. The productions of LMW met^5-enk like and LMW leu^5-enk like were inhibited by soybean trypsin inhibitor and N-α-p-tosyl-lysyl-chloromethylketone respectively. The effectiveness of these trypsin inhibitors suggests that some of the enzymes in the biosynthesis of enkephalins may be trypsin-like in their substrate specificity.

Project Description:

Several lines of evidence suggest that enkephalins are formed from high molecular weight precursors. Bovine adrenal glands was found to contain a very large quantity of enkephalin-like immunoreactive peptides especially the high molecular weight forms. Because of these observations, we have decided to study the biosynthesis of met^5- and leu^5-enkephalins in bovine adrenal chromaffin granules. A soluble protein fraction was prepared from lysed chromaffin granules by ammonium sulfate precipitation followed by dialysis. This protein preparation was found to produce low molecular weight (LMW) met^5- and leu^5-enkephalin like peptides when incubated at 37°C. Furthermore, the production of the LMW enkephalin like peptide appears to increase with the time of incubation at 37°C. The LMW enkephalin-like peptides produced were analyzed by HPLC and found to contain met^5- and leu^5-enkephalins and also other as yet unidentified immunoreactive peptides. The results clearly indicate that the granule preparation contains both the enkephalin generating enzyme(s) and precursor(s) of enkephalins. In order to further characterize the enkephalin generating enzyme(s), various trypsin inhibitors were tested. N-α-p-tosyl-lysyl-chloromethyl ketone and p-aminobenzamidine partially inhibited the production of leu^5-enkephalin immunoreactivity but were without effect on the generation of LMW met^5-enkephalin immunoreactivity. The effect of these trypsin inhibitors suggests that enkephalin generating enzymes may be trypsin-like in their substrate specificity. We are currently continuing on the purification of the enkephalin generating enzymes and the identification of the endogenous precursors of enkephalins.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01554-01 SMRP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Met^5-enkephalin system in pituitary: Heroin addicts vs controls

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Govoni	Guest Worker	SMRP	NIMH
Other:	L. G. Harsing	Visiting Associate	SMRP	NIMH
	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	J. Kleinman	Staff Psychiatrist	SMRA	NIMH

COOPERATING UNITS (if any)

Adult Psychiatry Branch

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Very high contents of met^5-enkephalin-like peptides were found in pituitaries of many species if animals were killed by decapitation. Furthermore, met^5-enkephalin immunoreactivity in pituitary was found to consist of high (MW $\gtrsim$1800) and low (MW about 600) molecular weight enkephalin-like peptides. Interestingly, one of the high molecular weight forms was found to be much lower in pituitaries of heroin addicts in comparison to controls. The molecular mechanism underline this heroin induced modification in met^5-enkephalin immunoreactivity still remains to be established.

Project Description:

Originally, we found that pituitary glands of rats contained very low level of met^5-enkephalin immunoreactivity. Subsequently, a very high met^5-enkephalin content was reported in rat pituitaries by some investigators. In order to understand this discrepancy, we have compared the met^5-enkephalin immunoreactivity in pituitary glands of rats killed by focussed microwave irradiation and those killed by decapitation.

The pituitary content of enkephalin-like peptides was found to be at least 10 times higher in rats killed by decapitation than in rats killed by focussed microwave irradiation. Pretreatment of rats with pentobarbital exerted no effect on the pituitary met^5-enkephalin immunoreactivity. The results suggest that the high met^5-enkephalin immunoreactivity observed in decapitated rats is not due to stress induced by decapitation. Furthermore, analysis of the pituitary enkephalin immunoreactivity revealed the presence of many different molecular forms of enkephalin like peptide. The high molecular weight forms (MW $\gtrsim$ 1800) was found to generate met^5-enkephalin when treated with trypsin. High levels of met^5-enkephalin immunoreactivity were observed in pituitaries of many species, such as rats, mice, guinea pigs and hamsters, if they were killed by decapitation.

In human, we have compared the pituitary enkephalin immunoreactivity of heroin addicts with that of controls. The total immunoreactivity was found to be lower in addicts than in controls. This reduced level was found to be due to one of the high molecular weight forms. Interestingly, met^5-enkephalin content was found to be similar in the addicts and controls. Although the result indicated that, in man, opiate addiction modified the content and composition of enkephalin-like peptides in pituitary glands, but how and why this comes about still remains to be established.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01555-01 SMRP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Enkephalin metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	An-Zhong Zhang	Visiting Fellow	SMRP	NIMH
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	E. M. Majane	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.05	1.05	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to study the role of dipeptidyl carboxypeptidase in enkephalin metabolism, a new enzyme assay method, using [DNS][^{3}H]-Gly-Gly-Phe-Leu as substrate, was developed. The enkephalin inactivating dipeptidyl carboxypeptidase (enk inact. DPC) was inhibited markedly by acethiorphan but not by angiotensin converting enzyme inhibitor SQ 20881 or captopril. The regional distribution of enk inact. DPC in the brain parallels closely with that of enkephalins except in the region of cerebellum. Met^5-enkephalin-Arg^6-Phe^7 was readily converted to met^5-enkephalin also by a dipeptidyl carboxypeptidase. This converting activity was inhibited by the angiotensin converting enzyme inhibitor SQ 20881 but not by acethiorphan. With the selective inhibitors of the dipeptidyl carboxypeptidase, we are currently studying the physiological role of the dipeptidyl carboxypeptidase, in the metabolism of enkephalins and met^5-enkephalin-Arg^6-Phe^7.

Project Description:

Enkephalin is known to be very rapidly degraded in tissues by proteases such as aminopeptidase, dipeptidyl aminopeptidase and dipeptidyl carboxypeptidase. Recently accumulated evidence suggests that physiological inactivation of enkephalin may be mediated by a dipeptidyl carboxypeptidase. In order to explore further the role of dipeptidyl carboxypeptidase in enkephalin metabolism, we have 1) developed a simple and sensitive method to assay the enkephalin inactivating dipeptidyl carboxypeptidase (enk inact. DPC) in brain; 2) studied the metabolism of other enkephalin-like peptide, met^5-enkephalin-Arg^6-Phe^7 and 3) initiated a study on the effect of the dipeptidyl carboxypeptidase inhibitors on the endogenous level of enkephalins and met^5-enkephalin-Arg^6-Phe^7.

The enk inact. DPC was inhibited efficiently by o-phenanthroline and acethiorphan and moderately by Phe-Ala. On the other hand, known inhibitors of angiotensin converting enzyme SQ 20881 and captopril exerted totally no effect on the enk inact. DPC. The regional distribution of the enzyme in rat brains was studied with [DNS][^{3}H]-Gly-Gly-Phe-Leu as substrate. The highest enzyme activity was found in the striatum and the lowest in hippcampus. The distribution of enk inact. DPC seems to correlate well with enkephalin distribution except in cerebellum. In the cerebellum, there is a very low level of enkephalins but a high concentration of the enzyme.

The newly characterized enkephalin-like peptide, met^5-enkephalin-Arg^6-Phe^7 was readily converted to met^5-enkephalin by a dipeptidyl carboxypeptidase. Furthermore, met^5-enkephalin-Arg^6-Phe^7 was hydrolyzed at a much higher rate than enkephalins by the dipeptidyl carboxypeptidase. Interestingly, the angiotensin converting enzyme inhibitor SQ 20881 inhibited the conversion of met^5-enkephalin-Arg^6-Phe^7 to met^5-enkephalin efficiently while it showed little effect on met^5-enkephalin hydrolysis. Chloride ion, which is required for angiotensin converting enzyme, did not activate the conversion of met^5-enkephalin-Arg^6-Phe^7 to met^5-enkephalin. Whether there is a specific dipeptidyl carboxypeptidase for the conversion of the heptapeptide to met^5-enkephalin remains to be established by a purified enzyme. Currently we are continuing to study the effect of the dipeptidyl carboxypeptidase inhibitors on the endogenous levels of met^5-enkephalin and met^5-enkephalin-Arg^6-Phe^7.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01556-01 SMRP

PERIOD COVERED

October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Release of Endorphin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Harsing	Visiting Associate	SMRP	NIMH
Other:	H.-Y.T. Yang	Pharmacologist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Release of enkephalin from striatum was studied by perfusion of tissue slices. Depolarizing concentration of KCl (47 mM) elicited an increase of met^5-enkephalin (ME) efflux during the perfusion. The increased rate of ME efflux induced by high K^+ is Ca^{2+} dependent and could be further increased by the addition of enkephalin inactivating dipeptidyl carboxypeptidase Phe-Ala. Micromolar concentrations of diazepam and flunitrazepam failed to change the basal rate of ME efflux but inhibited in a concentration dependent manner the high K^+ induced efflux of ME. This action of benzodiazepine was mimicked by muscimol and THIP but antagonized by picrotoxin. When the slices were prepared from striatum of rats receiving high doses of isoniazid, which reduced GABA content by about 60% diazepam but not muscimol failed to reduce the K^+ induced ME efflux. The study indicates that the inhibition of ME efflux during depolarization elicited by diazepam is dependent on endogenous GABA and GABA receptors. Effect of anoretic drugs on endorphin system was also investigated in this study Chronic-treatment of rats with d-fenfluramine increased the hypothalamic contents of met-enkephalin and β-endorphin. Whether the anoretic action is mediated by decrease utilization of ME and β-endorphin will be investigated by release study.

Project Description:

In this study, release of enkephalin from striatum was investigated by a superfusion technique. Inclusion of 47 mM KCl in the perfusion medium increase the rate of met^5-enkephalin (ME) efflux by about 3 fold. This high K^+ induced ME efflux was abolished when Ca^{++} was omitted from the perfusion medium. The recovery of the released met^5-enkephalin was higher when Phe-Ala (0.1 mM) was added to the perfusion medium to inhibit the enkephalin inactivating dipeptidy carboxypeptidase. Diazepam, flunitrazepam, muscimol, THIP and baclofen were found to reduce the increased ME efflux elicited by high K^+ On the other hand, these drugs seemed to have no effect on the basal rate of ME efflux. The rank order of the potency of these drugs were muscimal>flunitrazepam>diazepam≈THIP≈baclofen. Picrotoxin failed to change the ME release induced by KCl, while it completly reversed the diazepam inhibition of the increase in ME efflux elicited by KCl. Treatment of rats with high dose of isoniazid (450 mg/kg) reduced the GABA content of the struatum to about 60% the control. When striatal slices were prepared from these isoniazid treated animals, diazepam but not muscimol failed to reduce the efflux of ME elicited by high K^+. The results suggest that the effect of diazepam is dependent on the certain amount of endogenous GABA, another words, the inhibition by diazepam of the release of ME caused by depolarization is mediated by GABA system.

It has been suggested that endorphin system may be involved in eating behaviour. In order to test this hypothesis, effect of d-fenfluramine and d-amphetamine on β-endorphin (β-E) and enkephalin (ME) was studied. The hypothalamic M'E content was elevated from 6.2±1.0 ng/mg protein to 12.9±0.9, 10.3±1.1 and 9.6±0.7 ng/mg protein on the 5th, 10th and 15th day of d-fenfluaramine treatment. This increase of ME content was associated with a marked decrease of body weight. A small increase of ME content was also observed in striatum on 5th day but not on 10th or 15 day of d-fenfluramine treatment. No changes in ME content were observed in other areas of brain such as frontal cortex, hippocampus, brain stem. The content of β-enkephin immunoreactive peptide was increased from 4.4±0.8 to 9.3±1.8 and 7.0±0.8 ng/mg protein on the 5th and 10th day of d-fenfluramine treatment. In contrast to d-fenfluramine, d-amphetamine exerted no effect on ME or β-E immunoreactive peptides in the brain. Whether the anoretic action is mediated yb decrease utilization of ME and β-endorphin will be investigated by release study.

5-0112

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